Instrumental Analysis and Quantitation of

Polycyclic Aromatic Hydrocarbons

In

Air and Precipitation Samples

Integrated Atmospheric Deposition Network Project

Prepared by
Karen Arnold
Yuning Ma
Kevin Romanak
Marta Venier
Amina Salamova

School of Public and Environmental Affairs
Multi-Science Building 2
Indiana University
Bloomington, Indiana 47405

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I. Introduction

This document outlines the instrumental analysis and quantitation of polycyclic aromatic hydrocarbons (PAH) measured in atmospheric (vapor and particle phases) and precipitation samples collected at six sites on the Great Lakes. This work is conducted as a part of the Integrated Atmospheric Deposition Network (IADN) at the School of Public and Environmental Affairs, Indiana University-Bloomington. This research is supported by the Great Lakes National Program Office of the U.S. Environmental Protection Agency.

PAH analyses are performed on a Hewlett-Packard (HP) 6890 gas chromatograph coupled with an HP 5973 mass spectrometer (GC/MS) operated in the electron ionization (EI) mode ion source (see Appendix I for method details on page 28).

Hewlett Packard GC 6890 model # G1513A and serial # US00008627
Mass Selective Detector 5973 model # G1099A and serial # US72020797
GC Autosampler Controller model # G1512A and serial # US72202110

Chromatographic resolution is achieved with a 30 m × 250 µm x 0.25 µm film thickness HP-5MS UI capillary column (J & W Scientific, Folsom, CA) with helium carrier gas. Previously we used DB-5MS capillary column with film 0.25 µm film thickness. PAH are analyzed by GC-MS using selected ion monitoring (SIM) and quantitated using the method of internal standards.

Quantitation is performed by Enviroquant using the method of internal standards. Relative response factors (RRFs) for each analyte are determined from the calibration standard's peak areas using equation 1:

$$\mathrm{RRF_{std}} = \left(\frac{\mathrm{mass_a}}{\mathrm{area_a}}\right)_{\mathrm{std}} \div \left(\frac{\mathrm{mass_{istd}}}{\mathrm{area_{istd}}}\right)_{\mathrm{std}} \qquad (1)$$

where $mass_a$ is the analyte's known mass in the injected amount of calibration standard, $area_a$ is the analyte's peak area, $mass_{istd}$ is the known mass of the appropriate internal standard, and $area_{istd}$ is that internal standard's peak area. With reference to Table I, the response factors for compounds 2-7, 11-12, and 14-21 are calculated relative to the internal standards d10-anthracene, d12-benz*[a]*anthracene, and d12-perylene, respectively.

An analyte's mass in a sample ($mass_a$) is calculated from the RRF_{std} above and the internal standard response in the sample by the following equation:

$$\left(\text{mass}_{\text{a}}\right)_{\text{sample}} = \left(\text{area}_{\text{a}}\right)_{\text{sample}} \times \text{RRF}_{\text{std}} \times \left(\frac{\text{mass}_{\text{istd}}}{\text{area}_{\text{istd}}}\right)_{\text{sample}} \qquad (2)$$

where $area_a$ is the analyte's peak area in the sample, $mass_{istd}$ is the mass of internal standard spiked into the sample, and $area_{istd}$ is the internal standard's peak area in the sample. The analyte concentrations are tabulated by Enviroquant and transferred to an Excel spreadsheet.

The PAH analyzed in this study are listed in Table I along with the quantitation and confirmation ions.

Table I: Target compounds and their monitored ions

Retention Order	Compound	Quantitation Ion	Confirmation Ion
1	d10-Anthracene **(IS)**	188	189
2	Fluorene	166	165
3	d10-Phenanthrene **(SS)**	188	189
4	Phenanthrene	178	176
5	Anthracene	178	179
6	Fluoranthene	202	203
7	Pyrene	202	203
8	d10-Pyrene **(SS)**	212	213
9	Retene	219	234
10	d12-Benz*[a]*anthracene **(IS)**	240	241
11	Benz*[a]*anthracene	228	226
12	Chrysene+ triphenylene	228	226
13	d12-Perylene **(IS)**	264	260
14	Benzo*[b]*fluoranthene	252	253
15	Benzo[*k]*fluoranthene	252	253
16	Benzo*[e]*pyrene	252	253
17	Benzo*[a]*pyrene	252	253
18	Indeno*[1,2,3,cd]*pyrene	276	277
19	Dibenzo *[a,h]*anthracene	278	279
20	Benzo *[g,h,I]*perylene	276	277
21	Coronene	300	301

II. Routine GC/MS Maintenance

1. Gas Tanks

Check the gas tank. It should not go dry. When the tank is at 500 PSI the tank should be replaced. While changing the tank lower the temperature of the GC oven to 40^0C. After replacing the tank, leave the temperature at 40^0C and turn the gas saver off for about 15 minutes to get rid of air or oxygen that was drawn in. Check for leaks using an electronic leak detector. Do not use a liquid leak check solution as it may be drawn into the instrument and may cause damage to the column.

2. Liner

The liner needs to be replaced when the response and resolution begins to lower. Turn down the injection port temperature to 30^0C. Wear clean, lint-free gloves to prevent contamination of parts with dirt and skin oils. Remove the insert weldment (inlet nut) so that the liner can be replaced. Replace with new liner and O-ring using tweezers. Re-install the column nut, finger tighten it, and then tighten it an addition ¼ turn. Turn off the gas flow saver so that the gas flows continuously for 5 minutes, then turn the gas saver back on.

3. Septum

The merlin septum needs to be replaced when necessary. Symptoms such as shifting retention times, loss of response, loss of column head pressure and/or increased signal noise indicate that the merlin septum needs to be replaced. Turn down the injection port temperature to 30^0C. Install new merlin septum and slowly finger tighten. Watch the gas pressure of the injection port until it becomes stable, and then tighten the septum about ½ turn. Put the tower back. If the pressure does not remain stable, take off tower and tighten a little more.

4. Gold Seal

The gold seal should be replaced when necessary. Turn down the temperature to 30^0C. Wear clean, lint-free gloves to prevent contamination of parts with dirt and skin oils. Remove the injector end of the column. Remove the insulation cup. Remove the reducing nut and replace the gold seal. After a new gold seal is installed tighten the reducing nut and then re-install the insulation cup. Re-install the column following the instructions for column installation and follow the procedures required after installing column.

5. Pump Oil

The pump oil should be replaced every 6 months. The used oil should be placed in a closed container labeled as hazardous waste and when full picked up by the IU Health and Safety Department for disposal.

6. Column

The column will need to be replaced or clipped if the peaks have bad shapes, show tailing, low responses, or begins to elute before 7 minutes.

Table II. Suggested maintenance check list (may vary according to instrument usage).

TASK	EVERY WEEK	EVERY 6 MONTHS	EVERY YEAR	AS NEEDED
Tune the MSD				•
Change injection port liners	•			
Check the foreline pump oil level	•			
Gas ballast the foreline pump	•			
Check the calibration vial		•		
Replace the foreline pump oil		•		
Check the diffusion pump fluid	•			
Replace the diffusion pump fluid			•	
Replace the traps and filters			•	
Clean the ion source				•
Change the carrier gas trap(s) and purifier				•
Replace the worn out parts				•
Lubricate seals (where appropriate)				•
Replace column				•

A. Installing a new column

1) Turn down the temperatures for GC, injection port, and the auxiliary (interface).

2) Go to the MSChem Station. Change to **View**, then click **Tune and Vacuum Control**, **Vacuum**, and then **Vent**.

3) When prompted (in approximately 40 minutes) turn off the MS.

4) Take off the cover of the instrument after the MS is turned off. **Be sure to wear the lint free gloves to do any work on the MS detector.**

5) Open the vent valve to break the vacuum. Once the instrument has vented close the valve (be sure not to tighten too much).

6) Unplug the cables (side board control cable and the source power cable) that are connected to the vacuum manifold.

7) Open the door of the vacuum manifold.

In the GC Oven:

8) Hang the column in the center of the GC oven to insure the column is uniformly heated during operation.

9) Place a septum, a new column nut, and then a graphite/vespel ferrule on the injector end of the column. Remove about 2 cm from the end. Verify that the cut end is straight and not jagged using a magnifying glass. Slide the septum to place the nut and ferrule so that only 4 to 6 mm of the column is showing from the tip of the column nut. Slide the nut up the column to the inlet base and finger tighten the nut. Tighten the column nut an additional ¼ to ½ turn

10) Start carrier gas flow and place the other end of the column end into hexane to check for flow through the column. After confirming gas flow, allow the gas to flow through the column for about 15 minutes without heating the GC oven. This allows the column to bleed off and make for a better baseline after installing a new column.

11) Condition column by ramping oven temperature starting at 30^0C and by increasing by 5^0C per minute to 300^0C and hold for 60 minutes. Total run time will be 115 minutes.

Table III. GC oven temperature and rates for column conditioning

Initial Temperature: 30ºC Initial Time: 1.00 min		
Rate (ºC/min)	**Final Temperature (ºC)**	**Final Hold Time (min)**
5.0	300	60.00
Total Run Time: 115 min		

12) Place a septum, a new column nut, and a graphite/vespel ferrule on the end of the column that will go to the MS portion. Cut 2 cm off of the end of the column. Verify that the cut end is straight and not jagged using a magnifying glass. Slide the column into the GC/MS interface until the column can be pulled through the vacuum manifold. Then adjust the column so that 1-2 mm projects past the end of the GC/MS interface and finger tighten the nut. Tighten the column nut an additional ¼ to ½ turn.

13) Close the vacuum manifold and make sure the vent valve is closed.

14) Go to MSChem Station then go to **View**, **Tune and Vacuum Control** and **Pump Down**. Hold the door with one hand and turn on the MS with the other. After a few seconds the vacuum will seal the door enough so you can release the hand from the door. The software will prompt you to turn on the GC/MS interface heater and GC oven. Click **OK**.

15) Re-install the MSD top cover. Make sure the temperature increases towards the set points. Allow the MSD to reach thermal equilibrium (it should be left to equilibrate overnight; if that's not possible, the minimum is 4 hours).

16) After allowing the instrument to equilibrate check for air and water by going to **Parameters**, **Manual Tune**, **More Parameters**, and then **Tune Parameters**. Make sure the parameters show Mass 1= 69.0, Mass 2= 32.00, and Mass 3= 18.00. Click okay. The air and water should be 1% and under for the instrument to be free from air and water.

17) Auto tune the instrument with perfluorotributlamine (PFTBA) calibration gas. Go to **Instrument #1** and then to **Instrument #1 MSTop/Enhanced**. Click on the **View** file and select **Tune and Vacuum Control**. Select **Tune** and then **Autotune**, the instrument will automatically select the parameters for the best working conditions. Once the instrument has been tuned be sure to save the autotune file (save it under the same name to replace the previous autotune file).

18) Run a hexane blank and a calibration standard to verify the peaks and response. Check the column nut to make sure that it is still tight.

19) In the SIM mode check the regions for each mass assignment. When the column is replaced or clipped, the retention times will shift. Make adjustments if necessary.

B. Clipping Column

The PAH calibration standard and the PAH common reference standard should be monitored to check for peak detection and response, and for peak broadening or tailing. If the peak shapes are not satisfactory, consider the following items:

- check the quantity / quality of the samples run after the most recent ion cleaning to verify that the ion source is not dirty and clean if necessary
- check the liner and replace if necessary
- check the syringe to verify that it is not blocked and is injecting properly and replace if needed
- if the peak response and shape is still not good then the column should be clipped.

1) Turn temperature down to 30^0C. Open the GC oven and remove the column from the injector end. Cut just behind the septum so that a new septum, new column nut and a new graphite / vespel ferrule can be slid onto the column before clipping. If the column is contaminated, at least 30 cm of the column length should be clipped.

2) Place a new septum, a new column nut, and a graphite/vespel ferrule on the injector end of the column. Remove about 2 cm from the end. Verify that the cut end is straight and not jagged using a magnifying glass. Slide the septum to place the nut and ferrule so that only 4 to 6 mm of the column is showing from the tip of the column nut. Slide the nut up the column to the inlet base and finger tighten the nut. Tighten the column nut an additional ¼ to ½ turn.

3) Return temperatures according to operating procedures. Allow the gas to flow through the column for 5 minutes to push out any contaminates in the column.

4) Check for any air and water in the instrument by following the procedure mentioned in installing a column.

5) Auto tune the instrument with perfluorotributlamine (PFTBA) calibration gas after allowing the instrument to set overnight. Go to **Instrument #1** and then to **Instrument #1 MSTop/Enhanced**. Click on the **View** file and select **Tune and Vacuum Control**. Select **Tune** and then **Autotune**, the instrument will automatically select the parameters for the best working conditions.

6) After tuning is finished select **File** and then **Save tune values**. A print out of the tune report will be generated automatically. If peak shapes in the printout look jagged or if some parameters are significally different from the previous tune values, check the installation of the ion source, gas leakage, or consult an Agilent technician.

7) Run a hexane blank to check for contamination. Check the column nut after this run and tighten if necessary.

8) Run a calibration standard to verify peaks and response.

9) Check the region for each mass assignment using the SIM mode.

C. Ion Source Cleaning

The frequency of cleaning the ion source depends on the number and type of samples being run through the instrument. Check column response and peak shape to determine whether the ion source needs cleaning. Removing contaminants restore the electrostatic properties of the ion source lensing system. **When handling the ion source be sure to use lint free gloves.**

1. Materials needed to clean ion source:

Abrasive paper
Alumina abrasive powder
Aluminum foil
Cotton swabs
Beaker
Gloves, Nylon lint-free and nitrile
Acetone
Methanol
Methylene chloride
Ultrasonic bath

2. Removing the ion source

1) Go to the MSChem Station. Change to **View**, then click **Tune and Vacuum Control**, **Vacuum**, and then **Vent**.

2) When prompted (approximately 40 minutes) turn off the MS. **Be sure to wear the lint free gloves to do any work on the MS detector.**

3) Take off the cover of the instrument after the MS is turned off.

4) Open the vent valve to break the vacuum. Once the instrument has vented close the valve (be sure not to tighten too much, but also make sure it's not loose or leaking).

5) Unplug the cables (side board control cable and the source power cable) that are connected to the vacuum manifold.

6) Open the door of the vacuum manifold. Disconnect the 7 colored wires from the ion source. Disconnect the wires for the ion source heater and temperature sensor. Remove thumb screws from the ion source and place screws inside the MSD and close the door.

7) Pull the ion source out of the source radiator.

3. Disassembling the ion source

1) After ion source is removed from the source radiator, remove the filaments.

2) Remove the repeller.

3) Unscrew the interface socket.
4) Remove the setscrew for the lenses.

5) Push the lenses out of the source body.

4. Cleaning the ion source

1) Use nitrile gloves while cleaning the ion source.

2) After the ion source is disassembled wrap the pieces that will not be cleaned in a piece of foil until ready to reassemble ion source.

3) There are 7 pieces that should be cleaned (see Figure I):
   - Repeller
   - Draw out plate
   - Source body
   - Ion focus lens
   - Entrance lens
   - Draw out cylinder
   - Interface socket

Figure I. EI ion source parts to clean.

4) Place a small amount of aluminum powder in a beaker and add reagent grade methanol to make a slurry mixture. Use this slurry mixture and sand paper saturated with methanol to abrasively clean the parts listed above. Clean all the surfaces that come into contact with the sample or ion beam.

5) Rinse away all abrasive residues with reagent grade methanol. Make sure all abrasive residues are rinsed away before the ultrasonic cleaning. If the methanol becomes cloudy or contains visible particles, rinse again.

6) Place the parts in a clean beaker. Making sure to cover all parts with the solvent and loosely cover the beaker with clean aluminum foil. Ultrasonically clean the parts for 15 minutes in each of the following reagent grade solvents in the order listed below.

- Methylene Chloride
- Acetone
- Methanol.

7) Dry the cleaned parts by letting them to air dry in aluminum foil for 15 minutes (or longer if necessary). Make sure that the parts are covered by foil.

5. Reassembling the ion source

1) Be sure to use the lint-free gloves before assembling the ion source.

2) Slide the draw out plate and the draw out cylinder into the source body.

3) Assemble the ion focus lens, entrance lens, and lens insulators.

4) Slide the assembled parts into the body source.

5) Install the setscrew that holds the lenses in place.

6) Re-install the repeller, repeller insulators, washer, and repeller nut into the source heater assembly.

7) Reconnect the repeller assembly to the source body.

8) Re-install the filaments.

9) Re-install the interface socket.

6. Re-installing the ion source

1) Slide the ion source into the source radiator.

2) Install and hand tighten the source thumbscrews.

3) Reconnect the 7 colored wires to the appropriate pins in the ion source (see Figure II).

Wire color	**Connects to**
Blue	Entrance lens (far right pin)

Orange	Ion focus (left pin)
White	Filament 1 (top filament)
Red	Repeller (middle pin)
Black	Filament 2 (bottom filament)

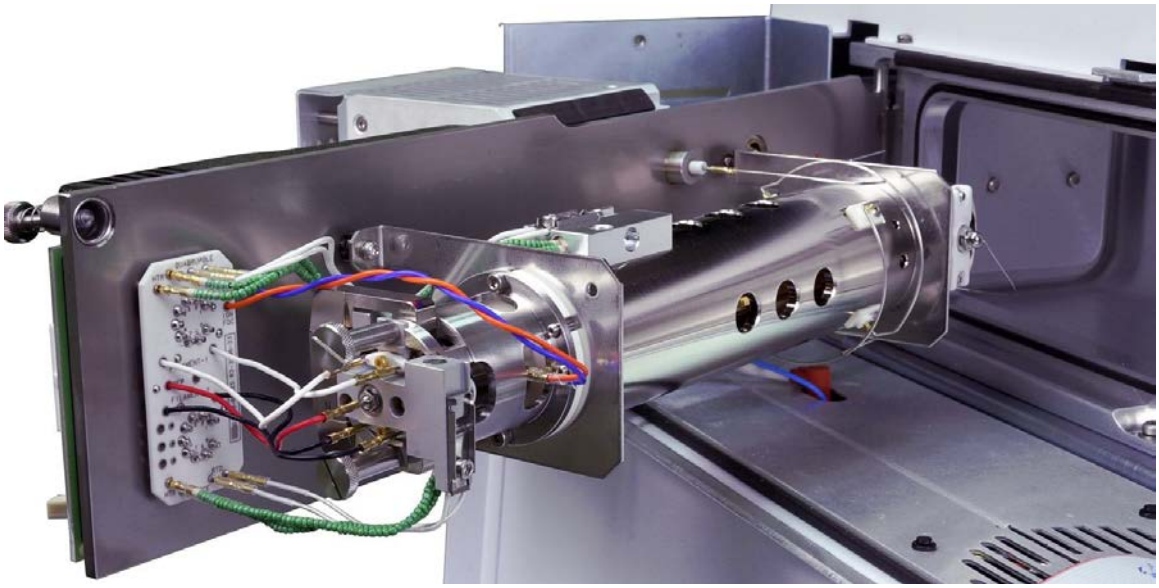


Figure II. EI ion source parts.

4) Reconnect the source heater and temperature sensor wires to the pins on the feed-through board.

5) Close the vacuum manifold.

6) Pump down the MSD.

7) Go to MSChem Station then go to **View**, **Tune and Vacuum Control** and **Pump Down**. Hold the door with one hand and turn on the MS with the other. After a few seconds the vacuum will seal the door enough so you can release the hand from the door. The software will prompt you to turn on the GC/MS interface heater and GC oven. Click **OK** after this is turned on.

8) Re-install the MSD top cover. Make sure the temperatures increase towards the set points. Wait at least 4 hours (usually overnight) for the MSD to reach thermal equilibrium and check for air and water.

7. Tuning the instrument on

1) After allowing the instrument to equilibrate, check for air and water by going to **Parameters**, **Manual Tune**, **More Parameters**, and then **Tune Parameters**. Make sure the parameters show Mass 1 = 69.0, Mass 2 = 32.00, and Mass 3 = 18.00. Click okay. The air and water should be 1% and under for the instrument to be free from air and water.

2) Auto tune the instrument with perfluorotributlamine (PFTBA) calibration gas after the instrument has equilibrated overnight or a minimum of 4 hours. Go to Instrument #1 and then to Instrument #1 MSTop/Enhanced. Click on the **View** file and select **Tune** and **Vacuum Control**. Select **Tune** and then **Autotune**, the instrument will automatically select the parameters for the best working conditions.

3) After tuning is finished select **File** and then **Save** tune values. A print out of the tune report will be generated automatically. If peak shapes in the printout look jagged or if some parameters are significally different from the previous tune values, check the installation of the ion source, gas leakage, or consult an Agilent technician.

4) Run a calibration standard to verify the peaks and response.

III. Routine GC/MS Operation

1. Instrument Parameters

The GC oven temperature program is given in Table IV. Other significant gas chromatographic parameters are:

Carrier gas:	helium (99.999 %; Praxair or Indiana Oxygen)
Injector:	Temp: 285^0C
	Pulsed Splitless, 25.0 psi
	Constant Flow, 1.5 mL/min
Injection volume:	1 µL
Transfer line:	280^0C

The mass spectrometer is operated in the electron ionization (EI) mode with ion source and quadrapole temperatures of 230ºC and 150ºC respectively.

Table IV. GC oven temperature program for PAH analysis

Initial Temperature: 70ºC
Initial Time: 3.00 min

Rate (ºC/min)	Final Temperature (ºC)	Final Hold Time (min)
30.0	280	6.00
30.0	300	10.0

Total Run Time: 26.67 min

2. MSD Pre-run

Hexane is injected prior to the running of a sample set to insure the system is free from contaminants or interfering peaks. The Relative Standard Deviation (RSD) between a calibration standard and a Common Reference Standard should be within 20%.

Sample injections and system maintenance are recorded in the appropriate laboratory logbooks located near the instrument.

3. Loading a method

1) Using the pull down menus, go to "**View**" and select "**Instrument control**"

2) Using the pull down menus, go to "**Method**" and select "**Load**", select a method from the list. Note that all method files have a .D extension (i.e. PAH.D).

3) By default, the software will prompt you to save changes to the existing method. Always select "**No**" (unless a desirable change has been made in the method, see section 5 below).

4) Once you've loaded a method, the "**Instrument Control Panel**" will be displayed.

5) The "Instrument Control Panel" has four editable user options buttons:

   Inlets: The injection port heater temperature may be changed here. The maximum temperature is 300°C. Do not exceed this temperature and do not change any other parameters.
   Columns: Typically, no user changes are required for routine analysis.
   Oven → Edit GC Oven: The oven temperature ramp rate may be edited here. The maximum ramp rate is 70°C/minute. Do not change the maximum oven temperature (325°C).
   MS → Edit MS SIM/Scan: The solvent delay time may be edited here. The solvent delay must not be set to less than 5 minutes. The mass range may be edited by pressing the "**Edit Scan Parameters**" button. The instrument mass upper limit is 800 amu.

   When finished, select from the pull down menus: **Method → Save**

3. Running the samples

1) Check if there is sufficient gas for operation. If not change the tank.

2) Open the door to the oven and check the column nuts to make sure they are tight.

3) Make the samples ready in microvials and prepare the MSD sequence.

4. Preparing the Sequence in MSD Chemstation

1) In the **"Instrument #1 MSTop/Enhanced"** view, go to **"Sequence"** and select **"Load"** to get an existing sequence or create a new one by using "default.s"

2) Go to **"Sequence"** and select **"Edit Sample Log Table"** to edit sequence parameters.

i. **Data Path:** select "**Browse"** and find your data directory. This is where the data will be stored.

ii. **Method Path:** select "**Browse"** and select from C:\msdchem\1\methods.

iii. **Sample Log Table:**

- Fill out Type (Sample, Blank, or Calibration), Vial (placement in autosampler tray), and Sample (name of the sample).

- Method/Keyword – Select box – a **"?"** will appear on right side of box. Click on the "?" and select the method to use.

- Data File – Select box – a "?" will appear on right side of box. Do not click on "?". Instead, double click in the box and a cursor will appear. Enter the name here and the computer will create the data file with that name. Be careful a duplicate file name may cause the new data overwrite the old data. To change the location of where the files are stored, go back up to Data Path and change it there.

- Check if all the vials number corresponds to their placement in the auto sampler tray.

- Click OK.

A typical sequence should have the following format: 3 hexane blanks, 1 calibration standard (see Table VI, page 20), common reference standard (CRS; see Table VII, page 21), 1 hexane blank, 8-9 samples (depending on how many samples are included in that specific set), and repeat needed. All injections are performed using a 6890 auto-injector with 1-µL injection volume. The run is completed with more hexane blanks and another calibration standard.

3) Save sequence (**Sequence → Save**).

4) Start sequence: Go to **"Sequence"** and select **"Load and Run Sequence".** A Start Sequence message box will appear.

- Select "Full Method".
- Click on the option for not overriding existing data.
- Enter your name in Operator Name box.
- Do not change Data File Directory.
- Click "**Run Sequence"**, instead of just clicking "OK".

- Go to **"Instrument #1"**. When prompted to override the solvent delay click "**No**" or leave it as it is.
- The run may be stopped at any time by clicking the red "**Stop**" button in the "**Instrument Control Panel**".

5) To monitor the run, click on the "Total Ion" thumbnail window in the "Instrument Control Panel" to enlarge the view. Click the "Instrument #1 Data Analysis" desk-top icon. From the pull down menus select File →Take Snapshot.

IV. Sample Analysis

The extracted samples, lab blanks and matrix spikes are stored in 4-mL amber vials at -20ºC until they are ready for analysis. These are the final extracts of 1-2 mL in hexane spiked with 50 µL of the internal standard solution (see Table V). Standards and samples are brought to room temperature before they are injected.

Table V. PAH internal standard solution.

Compound	Concentration (µg/mL)
d10-Anthracene	4.00
d12-Benz*[a]*anthracene	4.00
d12-Perylene	4.00

The mass spectrometer is turned on after a 6.0 minute solvent delay. Data is acquired in selected ion mode (SIM). Quantitation and confirmation ions and ion detection windows and are given in Table VIII, page 21.

V. Quality Assurance

Each analytical batch includes at least one calibration standard, one Common Reference Standard, one instrument blank, one procedure blank, and/or one matrix spike. Acceptance criteria are summarized on the attached table in the IADN Quality Assurance Project Plan (QAPjP,http://www.mscsmc.ec.gc.ca/iadn/resources/iu/Qapjp06.pdf).

Table VI. Calibration Standard

Retention Order	Compound	Concentration (µg/mL)
1	d10-Anthracene **(IS)**	0.20
2	Fluorene	0.20
3	d-10 Phenanthrene **(SS)**	0.20
4	Phenanthrene	0.20
5	Anthracene	0.20
6	Fluoranthene	0.20
7	Pyrene	0.20
8	d10-Pyrene**(SS)**	0.20
9	Retene	0.20
10	d12-Benzo*[a]*anthracene **(IS**)	0.20
11	Benz*[a]*anthracene	0.20
12	Chrysene+triphenylene	0.20
13	d12-Perylene **(IS)**	0.20
14	Benzo*[b]*fluoranthene	0.20
15	Benzo*[k]*fluoranthene	0.20
16	Benzo*[e]*pyrene	0.20
17	Benzo*[a]*pyrene	0.20
18	Indeno*[1,2,3,cd]*pyrene	0.20
19	Dibenz*[a,h]*anthracene	0.20
20	Benzo*[g,h,I]*perylene	0.20
21	Coronene	0.20

Table VII. Common Reference Standard

Retention order	Compound	Concentration µg/mL
2	Fluorene	0.20
3	d10-Phenanthrene **(SS)**	0.20
4	Phenanthrene	0.20
5	Anthracene	0.20
6	Fluoranthene	0.20
7	Pyrene	0.20
8	d10-Pyrene **(SS)**	0.20
9	Retene	0.20
11	Benz(a)anthracene	0.20
12	Chrysene+triphenylene	0.40
14	Benzo(b)fluoranthene	0.20
15	Benzo(k)fluoranthene	0.20
16	Benzo(e)pyrene	0.20
17	Benzo(a)pyrene	0.20
18	Indeno(1,2,3-cd)pyrene	0.20
19	Dibenz(a,h)anthracene	0.20
20	Benzo(g,h,i)perylene	0.20
21	Coronene	0.20

Table VIII. SIM windows and ions for analyte detection.

SIM Window	Start Time (min)	Ion Mass (m/z)
1	7.00	76, 83, 151, 152, 153, 154, 165, 166
2	8.50	101, 202, 176, 178, 188, 204, 212, 219, 234
3	10.60	114, 126, 252, 228, 240, 264
4	15.40	138, 139, 150, 276, 278, 279, 300

Note: The starting point of each region can change depending on the length of the column. Therefore, these starting times should be taken as reference only. Check calibration standard chromatogram for the peaks in the beginning of each region to properly adjust the window.

VI. Data Quantitation

1. Setting up a Quantitative Analysis

You will need to know what target and qualifier ions that are to be used for each compound. A **target ion** is an ion characteristic of the target compound, preferably one that distinguishes this compound from any other compounds with similar retention times. The extracted ion chromatogram for this ion will be used for quantitation. **Qualifier ions** are selected from the mass spectrum of the target compound. The presence of these ions in the correct amounts relative to the target ion gives evidence of correct target compound identification. Depending on the choice of peak selection parameters, the qualifiers may influence how the Chemstation software choses between multiple choices.

A) To create a quantitation method (Quant Database)

1) Load a calibration data file into Data Analysis. The TIC (total ion chromatogram) is automatically displayed.

2) Select **Calibrate/Set Up Quantitation**. Click **Ok**.

3) When the Edit Compounds box appears, click the position in the list of compounds where you want to insert a compound. This will allow you to associate a compound with an internal standard in the list. If the file is empty you are creating a new database, click **End of Compound List**. Then click the **Insert Above** button.

4) The Quant Setup box appears beneath the display of the TIC or EIC. You will use this box to select target ions and qualifiers for each compound that you want to add to the database. The Quant Setup box lets you use the mouse to automatically enter a compound's retention time and target/qualifier ion masses and ratios into the database.

   - Enter the name of the compound as you want it to appear in the database and reports.
   - Each compound in the database must have a unique name. Identical entries can cause compounds to be missed during quantitation.
   - Check the **ISTD** (internal standard) box if the compound is an internal standard.
   - Select the **Tgt** button. The remaining fields in the box will be selected and filled in according to mouse actions in the display.
   - Double-click the right mouse button on the scan in interest in the chromatogram. The selected spectrum will be displayed next to the Quant Setup box and the retention time will automatically be entered in the box.
   - The spectrum will be selected from the MS signal so the quantitation signal will be an MS quantitation ion.

- To select the target ion, move the mouse pointer to the target ion and click both mouse buttons at the same time. The target ion mass will be entered in the box and Q1 qualifier ion button will be selected.
- Click and hold the left mouse button and drag the mouse to create a rectangle around the ions of interest.
- To select the qualifier ion, click both mouse buttons on the ion. The mass will be entered in the box. Note the ratios to the target ion are also calculated and entered.
- Click the Save button to write the entry into the database.
- Repeat steps previous steps to add another compound.
- When you are finished adding compounds, click the **Exit** button.

5) The Edit compound box is automatically displayed in case you want to view or edit the compounds in the database.

6) Exit the compound box. To generate response data for a calibration curve, select **Calibrate/Update/Update One Level**.

7) Enter the concentration of the compound and the internal standard. Then select **Add New Level** and enter a **Calib Levels Update**. Click **Do Update**. The data file will be quantitated. If you are asked to re-quant now, answer **yes**.

8) The view or edit button will appear in case you want to edit the concentration and response data.

9) Save the database in a method. Be sure to do this before you leave the Data Analysis. If you do not the information just entered will be lost.

10) Select **Method** then **Save Method**. You may name to a new method or keep the current name if you are editing an existing method. Click **OK** to save. You will be reminded to save the changes and you will be ready to quantitate mass spectral data files containing unknown concentrations of one or more of the compounds in this database.

B) Quantitating Data

Use the quantitation method created according to the previous section (A) to quantitate a calibration standard. Update your method according to this calibration standard (see points 6-10 above). After the method is updated with the corresponding calibration standard values, the samples can be quantitated. The quantitation method can just be recalibrated from then on. The calibration method would not need to be set up again unless changes have been made to the calibration standard, such as new compounds. Each sample must be analyzed under the same conditions used for the calibration samples.

1. Loading a data file

- In Enhanced Data Analysis select **File** then **Load data file.**

- If loading a file from a new data set you will need to change the path to where the data set is located. If this is from a set that has been used you will just need to select the next data and click **ok**.

2. Loading a method file

- In Enhanced Data Analysis select **Method** then **Load Method**. You will be prompted to be sure that changes have been saved before loading method.

- Select method to use for quantitation from the list.

3. Quantitating data

a) In Enhanced Data Analysis select **Quantitate** then **Calculate**. You will see that this data has not been reviewed if it has not been quantitated. If it has already been quantitated you will see that this has been reviewed.

b) If you are reviewing it for the first time click on **View** then **QEdit Quant Result**. You will get several boxes one showing **Quick Edit**, the ion which will be the chromatogram being quantitated, TIC which will be the name of the compound being reviewed, and the scan which will be the Mass ratio.

a) Check the following:

1) Look at the peak shape. The peak shape in a sample should be similar to what is in the calibration standard. If the peaks are tailing, fronting, flat tops, split tops, or of any other irregular shape, refer to the GC Manual, page 70 for chromatographic problems and how to correct them. In some cases the sample needs to be rerun and / or recleaned. If these actions don't help then this compound needs to be deleted with a corresponding note in the data entry spreadsheets.

2) Look at the ion number in the scan and the TIC boxes. The quantitation ion is the molecular ion (M^+), and the confirmation ion is the second most abundant ion (see Figures from the chromatograph of the standard starting on page 34 for electron ionization mass spectrums of target PAH congener). The most abundant ion in the chromatogram and the quantitation ion in the TIC box should match. In addition, the confirmation ion in the chromatogram and in the TIC box should match.

There is an exception to this rule in case of deuterated PAH congeners and their parent compounds: anthracene and d10-antracene, pyrene and d10-pyrene, phenanthrene and d10-phenanthrene, and benzo[a]anthracene and d12-benz[a]anthracene. Due to peak overlapping of deuterated and parent compounds, the peaks of parent compounds could show the quantitation ion of the paired deuterated compound at a higher abundance than their characteristic quantitation ion. For example, phenanthrene and anthracene at low concentrations can show higher 188 than 178. However, ion 178 can't come from d-10 phenanthrene or d10 anthracene (see mass spectra pairs for deuterated and parent compounds in the Appendix, Figure VI). Therefore, in the case of these compounds the peaks ***should not be deleted*** *even if the most abundant ion is different from the quantitation ion. The same rule applies to benz[a]anthracene and pyrene. This is most likely to occur at low concentrations of the parent compound.*

3) Compounds Acenaphthlyene and Acenapthene are no longer reported for the IADN project. These compounds will be deleted.

4) Look at the response of the peak from the base of the peak and the top of the peak. The overall area (response) of the peak should be in at least 1:3 ratio to the baseline. For example, the base of the peak is at 100. The response of the peak is 800. It is more than 3 times higher than the base of the peak, so it is acceptable.

5) All peaks should have a straight quantitation baseline (shown in red). If it is not straight, adjust the baseline.

6) Look at the retention times. The unknown sample retention time in the TIC box should be close to the retention time in the calibration. Look at the numbers in the parenthesis (meaning how much the peak shifted from the calibration). It should be 0.010 or less. If the shift in the target peak is similar to that observed for the relative IS, then it is acceptable. However, if the peak shift is larger, then the sample might be too concentrated. Dilute and rerun again to confirm the peak. Use your judgment before deleting the peak.

4. Finishing quantitation of data and generating report

a) Click on **Exit** in the Quick Edit Box. You will be prompted to save the changes. Click **Yes**. This will change the screen to show the chromatogram in the scan and TIC boxes. The screen will also show that the data has been reviewed.

b) Select **Quantitate** then select **Generate Report**. A prompt box appears showing the report options. Select Summary for Style, and Print for Destination options.

Click **OK** to print out the report. Keep all the printouts in a folder designated for this batch of samples.

2. Creating a database for transferring data to Excel spreadsheets

a) Select **Quantitate** then **Custom Reports.** A prompt box labeled Control Panel will appear. Click on **Create New Database**.

b) A screen will show several things for reporting. The screen will show a division with one side labeled **Possible Items for Database** and the other side labeled **Database Contents**. From the list on the Possible Items for Database click on **Operator** and move to the Database Contents. Do the same for **Sample Name**, **Misc. Information**, and **Vial Number**.

c) Double Click on **Compound Information** from the Possible Items for Database and then double click on **All Compounds** on the Database Contents. From the possible items for the Database select **Amount** and move to the Database Contents. Click **OK**.

d) A screen will show Custom Reports stating that data analysis cannot be used while data files are being acquired. Click **Yes**.

e) A new screen will prompt a box titled Multiple File Select. This is where you can choose the files needed for reporting. The data files for the hexane blanks are not necessary for reports. From the Data File Name move the .d files to the Files Selected for Processing. When done click **OK**.

f) Save the report with the name of the set you are getting the .d files. You will be prompted with a new screen that links the method to the file. Click **OK**.

g) The next screen will prompt a box titled View Charts. Click **Close**. This will immediately go back to the Control Panel. Click **Exit**.

3. Entering the data into an Excel spreadsheet

a) Open Excel and open the data report. You will be prompted to make sure you want to open the file now. Click **Yes**.

b) A new screen will open. This will show the date of analysis, file name, operator name, sample name, misc. information, vial number, and the concentration of all compounds. This data can then be copied to the PAH initial data used for reporting.

4. Entering the data in IADN Raw data Entry spreadsheet

a) Open the PAH initial data spreadsheet and create a new empty tab.

b) Copy and paste a header from an existing tab into this new tab.

c) Copy the data set to be entered into this new tab.

d) Locate the PAH macro in the MACROS folder and open it.

e) Use PAH macro for the data to be entered (make sure you are at the new tab while using the macro).

f) Copy and paste the data to corresponding IADN Raw Data Entry spreadsheets. Save the changes.

g) Do not save the changes to the PAH initial data spreadsheets.

Appendix I. Instrument Control Parameters for PAH method.

INSTRUMENT CONTROL PARAMETERS
6890 GC METHOD

==========

OVEN
Initial temp: 70 'C (On) Maximum temp: 350 'C
Initial time: 3.00 min Equilibration time: 0.50 min
Ramps:

#	Rate	Final temp	Final time
1	30.00	280	6.00
2	30.00	300	10.00
3	0.0(Off)		

Post temp: 0 'C
Post time: 0.00 min
Run time: 26.67 min

FRONT INLET (SPLIT/SPLITLESS) BACK INLET (UNKNOWN)
Mode: Pulsed Splitless
Initial temp: 285 'C (On)
Pressure: 13.99 psi (On)
Pulse pressure: 25.0 psi
Pulse time: 2.00 min
Purge flow: 51.0 mL/min
Purge time: 2.00 min
Total flow: 55.0 mL/min
Gas saver: On
Saver flow: 20.0 mL/min
Saver time: 2.00 min
Gas type: Helium

COLUMN 1 COLUMN 2
Capillary Column (not installed)
Model Number: Agilent 122-5532
DB-5ms, 0.25mm * 30m * 0.25um
Max temperature: 350 'C
Nominal length: 30.0 m
Nominal diameter: 250.00 um
Nominal film thickness: 0.25 um
Mode: constant flow
Initial flow: 1.5 mL/min
Nominal init pressure: 14.09 psi
Average velocity: 45 cm/sec

Inlet: Front Inlet
Outlet: MSD
Outlet pressure: vacuum

FRONT DETECTOR (NO DET) BACK DETECTOR (NO DET)

SIGNAL 1
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 2
Use: MSD Transfer Line Heater
Description: MSD
Initial temp: 280 'C (On)
Initial time: 0.00 min

#	Rate	Final temp	Final time
1	0.0(Off)		

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

7673 Injector

Front Injector:

Sample Washes	0
Sample Pumps	3
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
Nanoliter Adapter	Off
PostInj Solvent A Washes	2
PostInj Solvent B Washes	2
Viscosity Delay	0 seconds
Plunger Speed	Fast

PreInjection Dwell 0.00 minutes
PostInjection Dwell 0.00 minutes
Sampling Depth -2.0 mm

Back Injector:
No parameters specified

Column 1 Inventory Number : USC321531H
Column 2 Inventory Number :

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : atune.u
Acquistion Mode : SIM

MS Information
-- -----------

Solvent Delay : 6.00 min

EM Absolute : False
EM Offset : 0
Resulting EM Voltage : 1776.5

[Sim Parameters]

GROUP 1
Group ID : 1
Resolution : Low
Plot 1 Ion : 76.0
Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(76.0, 10) (83.0, 10) (151.0, 10)
(152.0, 10) (153.0, 10) (154.0, 10)
(165.0, 10) (166.0, 10)

GROUP 2
Group ID : 2
Resolution : Low
Group Start Time : 8.80
Plot 1 Ion : 101.0
Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell) (Mass, Dwell)

(101.0, 20) (202.0, 20) (176.0, 20)
(178.0, 20) (188.0, 20) (204.0, 20)
(219.0, 20) (234.0, 20) (212.0, 20)

GROUP 3
Group ID : 3
Resolution : Low
Group Start Time : 11.00
Plot 1 Ion : 114.0
Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(114.0, 40) (126.0, 40) (252.0, 40)
(228.0, 40) (240.0, 40) (264.0, 40)

GROUP 4
Group ID : 4
Resolution : Low
Group Start Time : 16.40
Plot 1 Ion : 138.0
Ions/Dwell In Group (Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(138.0, 50) (139.0, 50) (150.0, 50)
(276.0, 50) (278.0, 50) (279.0, 50)
(300.0, 50)

[MSZones]

MS Quad : 150 C maximum 200 C
MS Source : 230 C maximum 250 C

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS

EMISSION : 34.610
ENERGY : 69.922
REPELLER : 31.296
IONFOCUS : 58.839
ENTRANCE_LE : 9.500
EMVOLTS : 1776.471
AMUGAIN : 1892.000
AMUOFFSET : 110.000
FILAMENT : 1.000
DCPOLARITY : 1.000
ENTLENSOFFS : 13.302

MASSGAIN : -178.916
MASSOFFSET : -12.589

END OF TUNE PARAMETERS

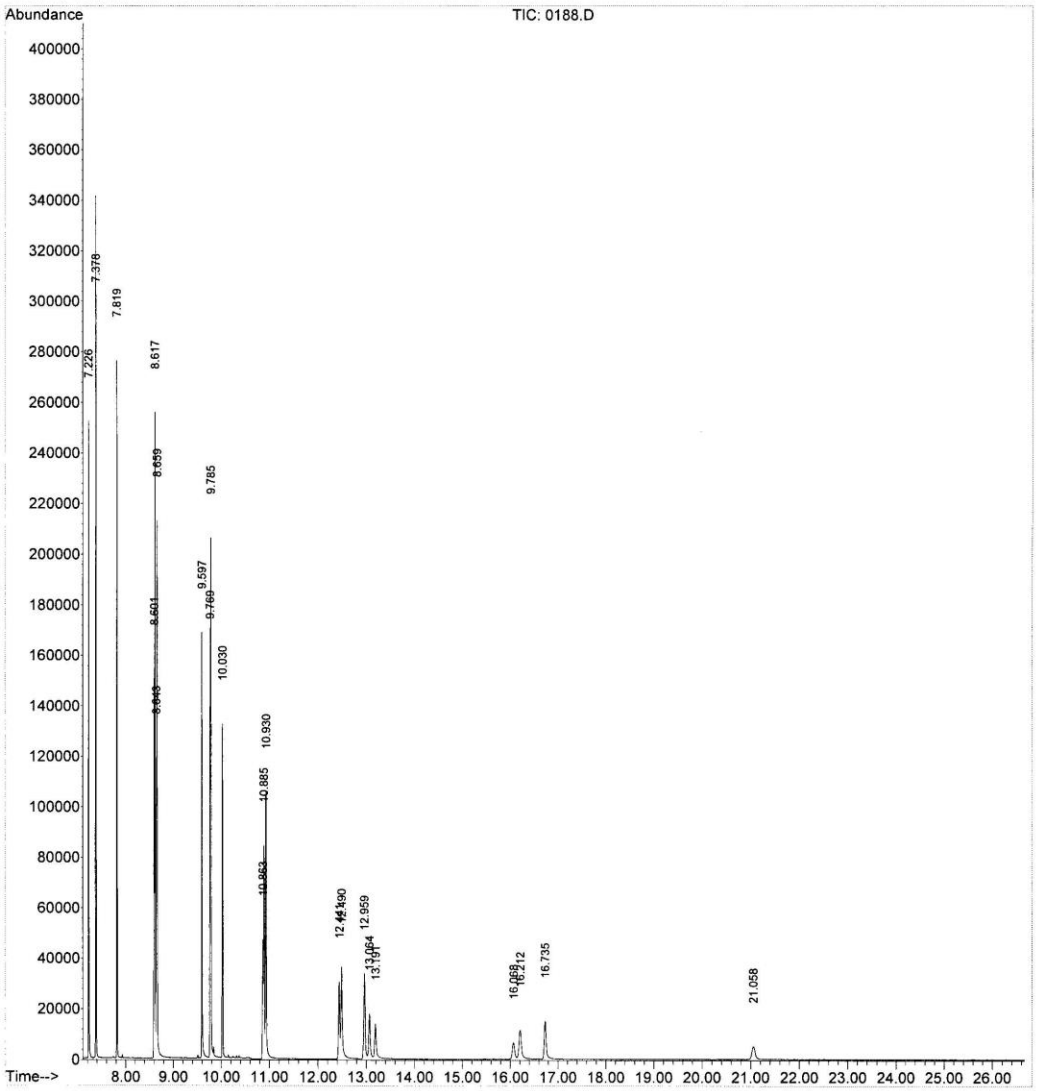
END OF INSTRUMENT CONTROL PARAMETERS

Figure III. An example of the chromatogram for calibration standard.

Quantitation Report (Not Reviewed)

Data Path : C:\MSDChem\1\DATA\
Data File : 0188.D
Acq On : 16 Jun 2009 18:07
Operator : YUNING
Sample : PAH CAL 200 ng/mL
Misc :
ALS Vial : 2 Sample Multiplier: 1

Quant Time: Jun 18 15:08:56 2009
Quant Method : C:\MSDCHEM\1\METHODS\PAH_NEWCUT_JUNE.M
Quant Title : IADN PAH
QLast Update : Thu Jun 18 15:08:53 2009
Response via : Initial Calibration



PAH_NEWCUT_JUNE.M Tue Aug 04 12:02:07 2009 Page: 2

Quantitation Report (Not Reviewed)

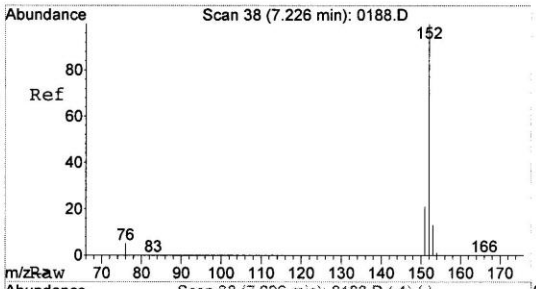
Data Path : C:\MSDChem\1\DATA\
Data File : 0188.D
Acq On : 16 Jun 2009 18:07
Operator : YUNING
Sample : PAH CAL 200 ng/mL
Misc :
ALS Vial : 2 Sample Multiplier: 1

Quant Time: Jun 18 15:08:56 2009
Quant Method : C:\MSDCHEM\1\METHODS\PAH_NEWCUT_JUNE.M
Quant Title : IADN PAH
QLast Update : Thu Jun 18 15:08:53 2009
Response via : Initial Calibration

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)
1) d-10 Anthracene	8.64	188	97284	200.00		0.00
12) d12-Dibenz(a)anthracene	10.86	240	57406	200.00		0.00
15) d12-Perylene	13.19	264	29218	200.00		0.00
System Monitoring Compounds						
5) d10-Phenanthrene	8.60	188	126167	200.00		0.00
Spiked Amount 200.000			Recovery = 100.00%			
10) d10-Pyrene	9.77	212	112685	200.00	ng	0.00
Spiked Amount 200.000			Recovery = 100.00%			
Target Compounds						Qvalue
2) Acenaphthylene	7.23	152	133127	200.00		100
3) Acenaphthene	7.38	154	87543	200.00		100
4) Fluorene	7.82	166	98904	200.00		100
6) Phenanthrene	8.62	178	156656	200.00		100
7) Anthracene	8.66	178	137969	200.00		100
8) Fluoranthene	9.60	202	121164	200.00		100
9) Pyrene	9.78	202	155744	200.00		100
11) Retene	10.03	219	47691	200.00		100
13) Benz(a)anthracene	10.89	228	81139	200.00		100
14) Chrysene + Triphenylene	10.93	228	116990	200.00		100
16) Benzo(b)fluoranthene	12.44	252	49411	200.00		100
17) Benzo(k)fluoranthene	12.49	252	60528	200.00		100
18) Benzo(e)pyrene	12.96	252	63023	200.00		100
19) Benzo(a)pyrene	13.06	252	33533	200.00		100
20) Indeno(1,2,3-cd)pyrene	16.07	276	19043	200.00		100
21) Dibenz(a,h)anthracene	16.21	278	24598	200.00		100
22) Benzo(g,h,i)perylene	16.73	276	36354	200.00		100
23) Coronene	21.06	300	20652	200.00		100

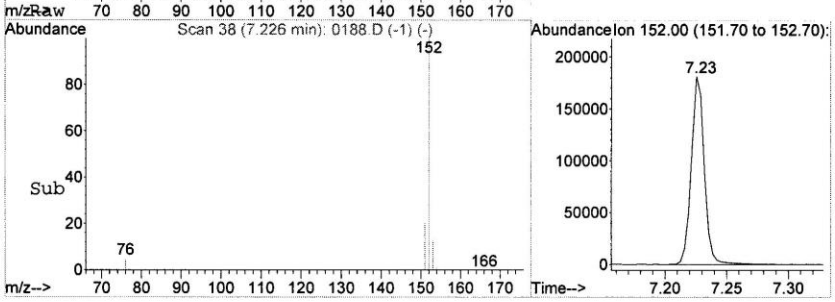
(#) = qualifier out of range (m) = manual integration (+) = signals summed

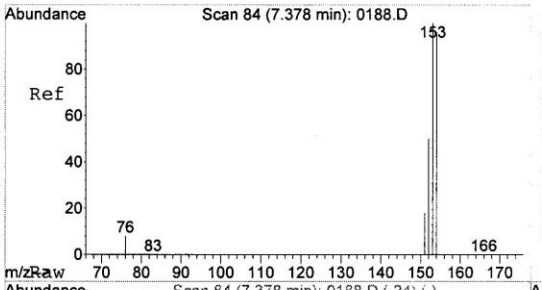
PAH_NEWCUT_JUNE.M Tue Aug 04 12:02:03 2009 Page: 1



#2
Acenaphthylene
Concen: 200.00
RT: 7.23 min Scan# 38
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:152 Resp: 133127

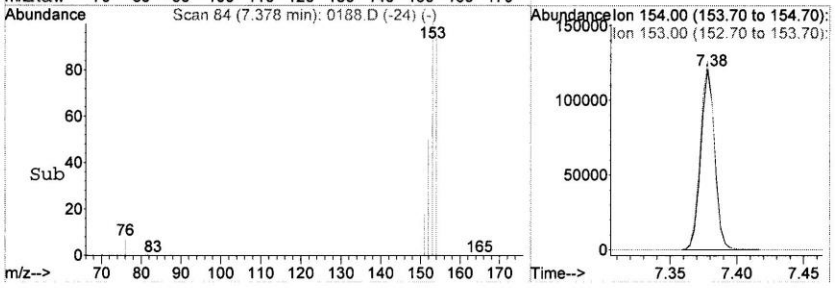


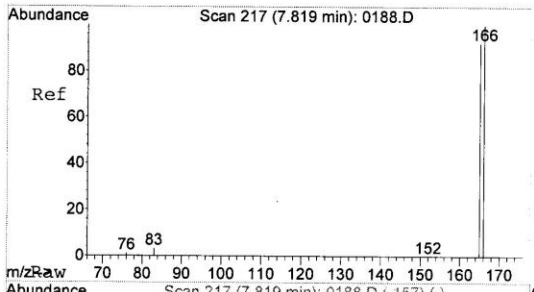


#3
Acenaphthene
Concen: 200.00
RT: 7.38 min Scan# 84
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:154 Resp: 87543

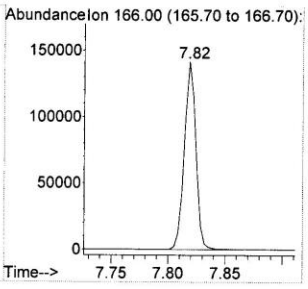
Ion	Ratio	Lower	Upper
154	100		
153	105.2	84.2	126.2

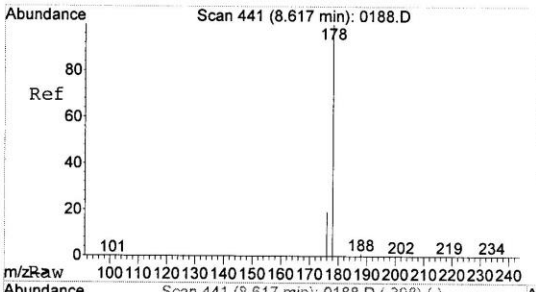




#4
Fluorene
Concen: 200.00
RT: 7.82 min Scan# 217
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

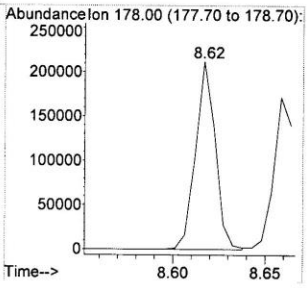
Tgt Ion:166 Resp: 98904

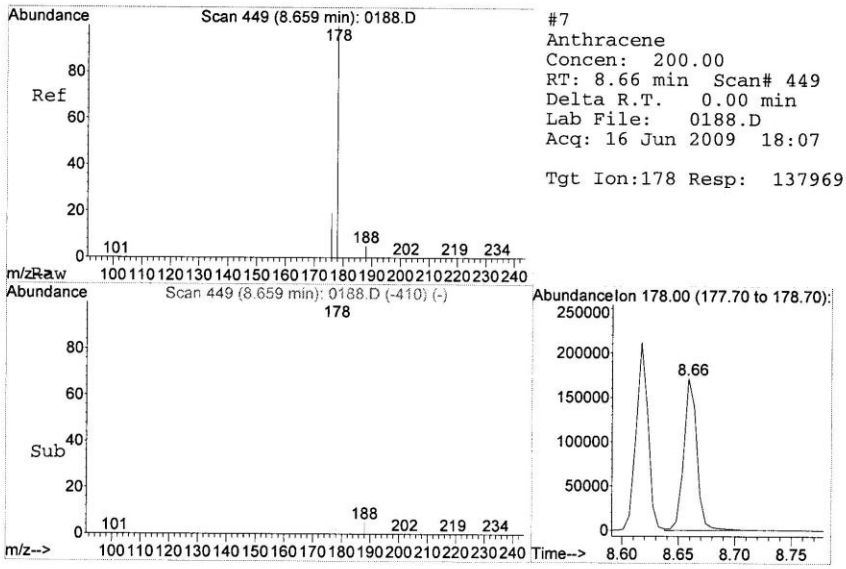




#6
Phenanthrene
Concen: 200.00
RT: 8.62 min Scan# 441
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

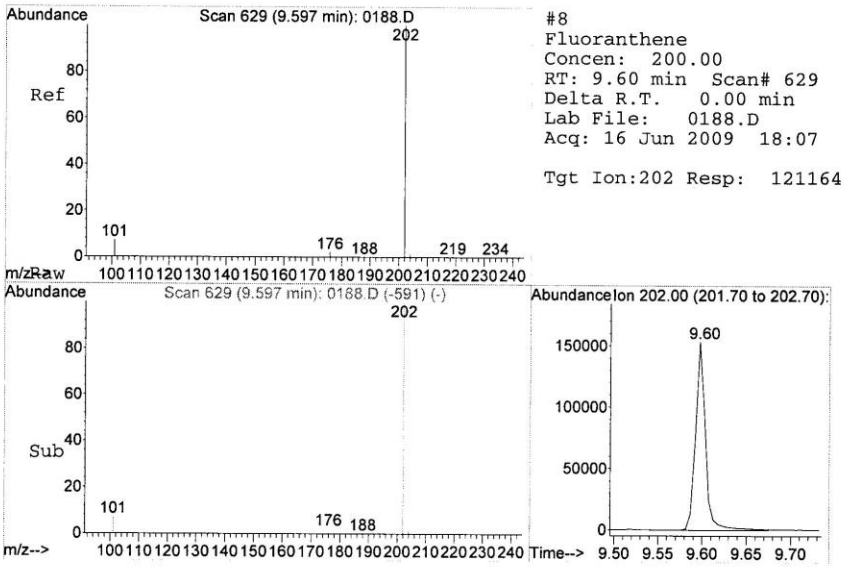
Tgt Ion:178 Resp: 156656





#7
Anthracene
Concen: 200.00
RT: 8.66 min Scan# 449
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

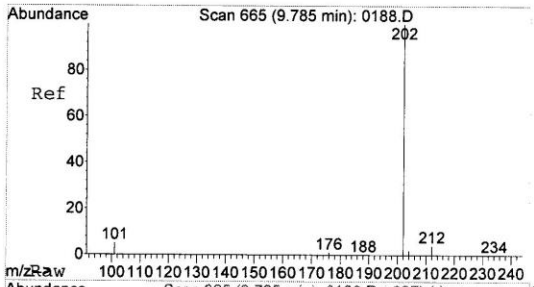
Tgt Ion:178 Resp: 137969



#8
Fluoranthene
Concen: 200.00
RT: 9.60 min Scan# 629
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

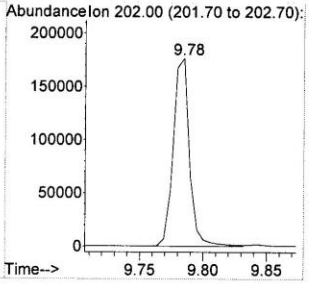
Tgt Ion:202 Resp: 121164

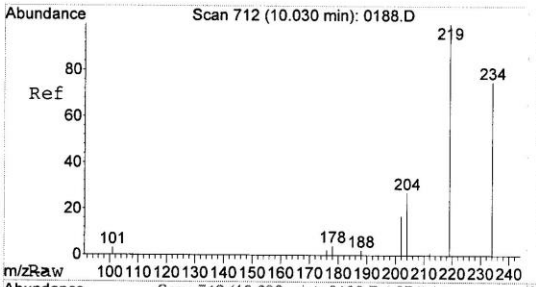
0188.D PAH_NEWCUT_JUNE.M Wed Aug 05 14:31:05 2009 Page 5



#9
Pyrene
Concen: 200.00
RT: 9.78 min Scan# 665
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:202 Resp: 155744

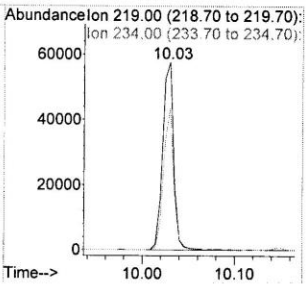


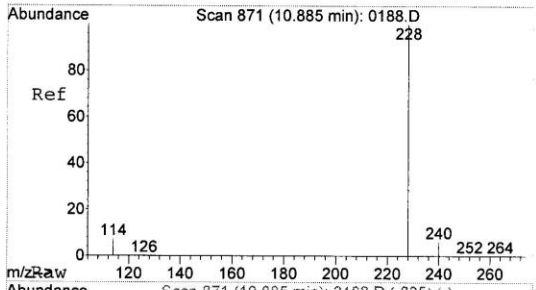


#11
Retene
Concen: 200.00
RT: 10.03 min Scan# 712
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:219 Resp: 47691

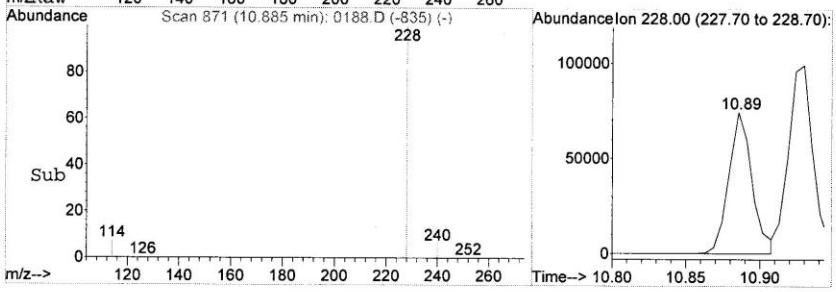
Ion	Ratio	Lower	Upper
219	100		
234	70.9	35.4	106.3

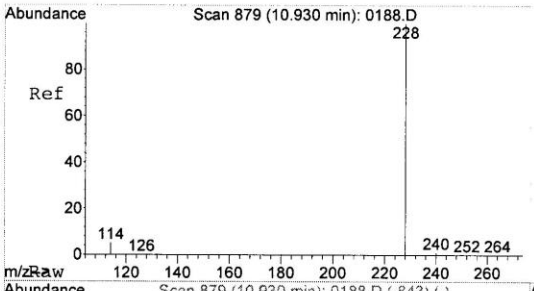




#13
Benz(a)anthracene
Concen: 200.00
RT: 10.89 min Scan# 871
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

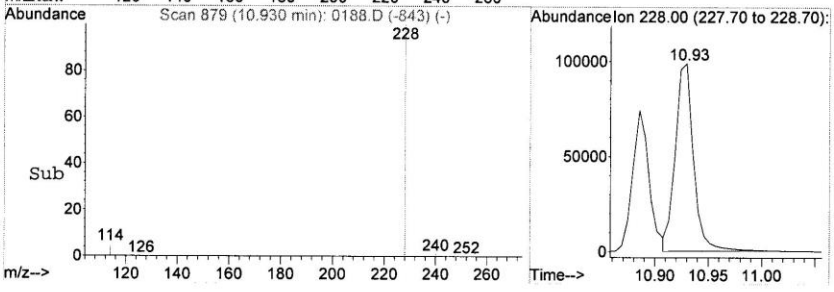
Tgt Ion:228 Resp: 81139

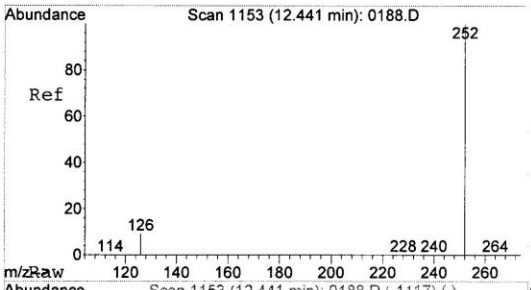




#14
Chrysene
Concen: 200.00
RT: 10.93 min Scan# 879
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

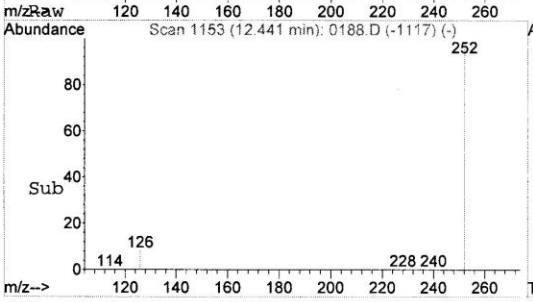
Tgt Ion:228 Resp: 116990

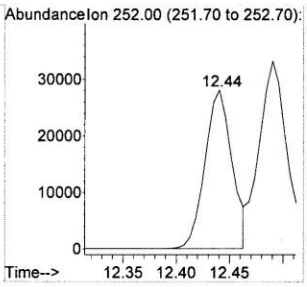


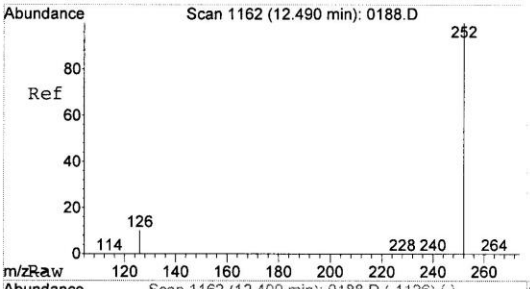


#16
Benzo(b)fluoranthene
Concen: 200.00
RT: 12.44 min Scan# 1153
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:252 Resp: 49411

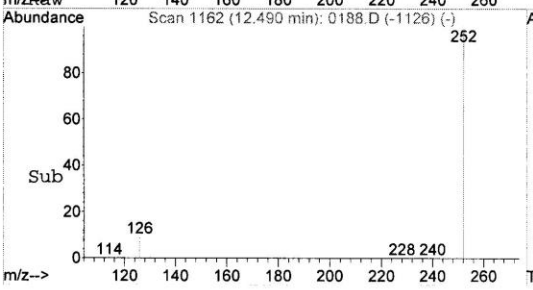


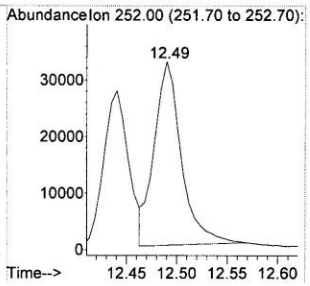


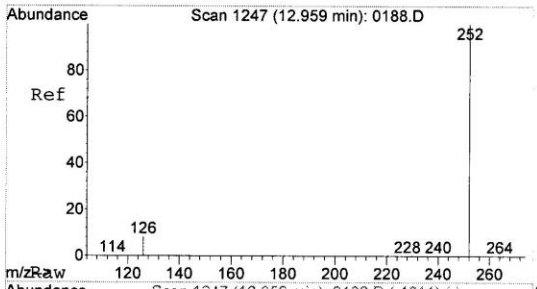


#17
Benzo(k)fluoranthene
Concen: 200.00
RT: 12.49 min Scan# 1162
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:252 Resp: 60528

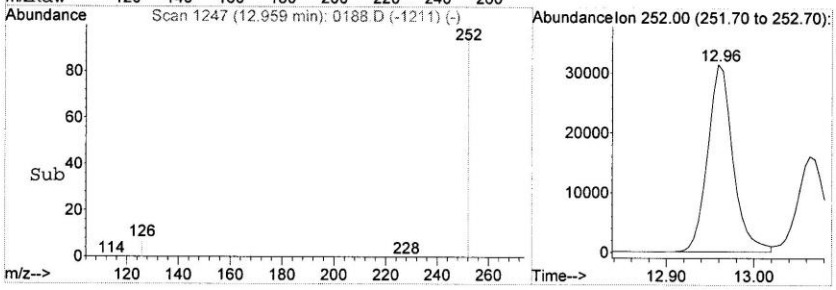


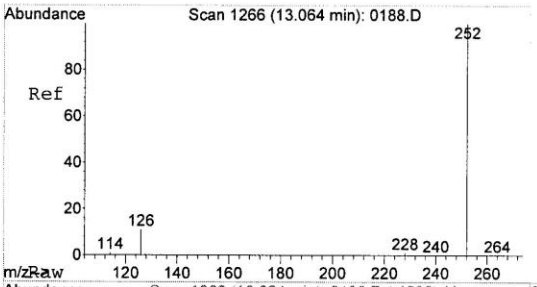




#18
Benzo(e)pyrene
Concen: 200.00
RT: 12.96 min Scan# 1247
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

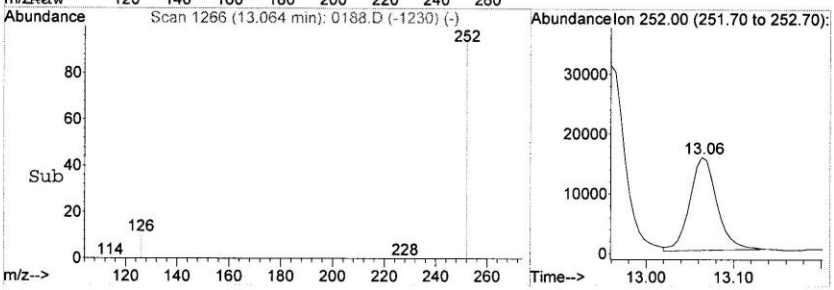
Tgt Ion:252 Resp: 63023

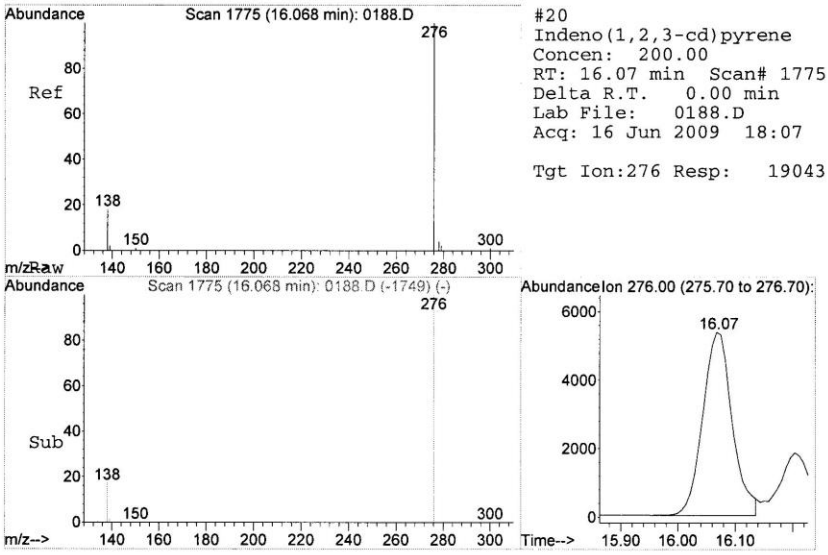




#19
Benzo(a)pyrene
Concen: 200.00
RT: 13.06 min Scan# 1266
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

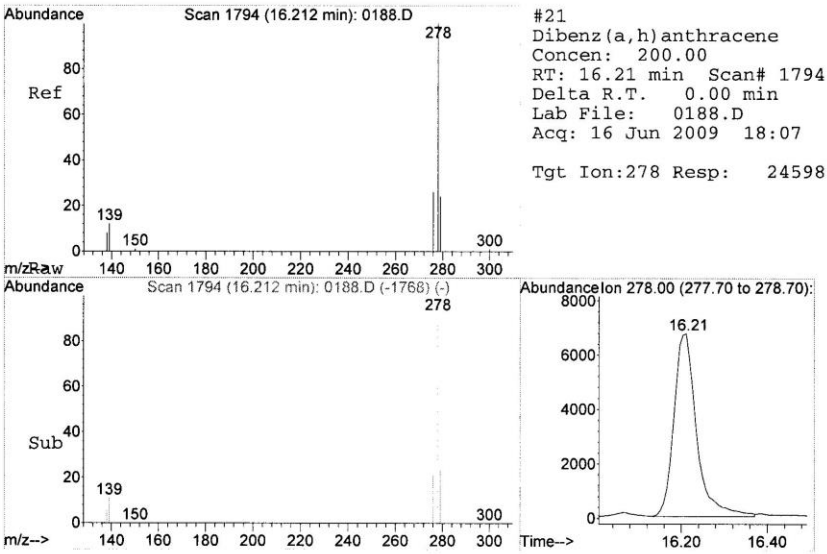
Tgt Ion:252 Resp: 33533





#20
Indeno(1,2,3-cd)pyrene
Concen: 200.00
RT: 16.07 min Scan# 1775
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:276 Resp: 19043



#21
Dibenz(a,h)anthracene
Concen: 200.00
RT: 16.21 min Scan# 1794
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:278 Resp: 24598

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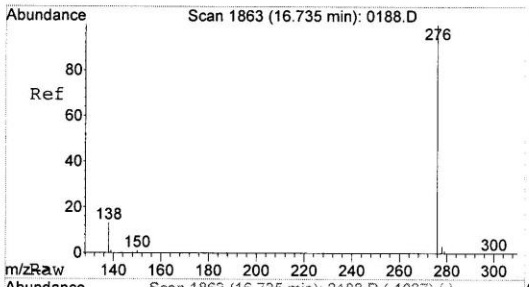
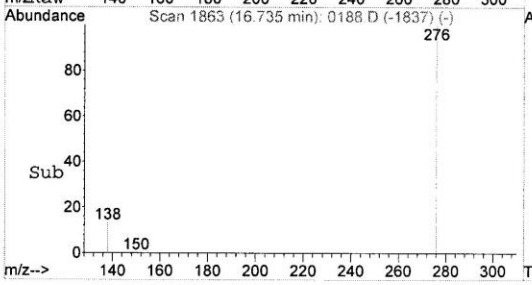
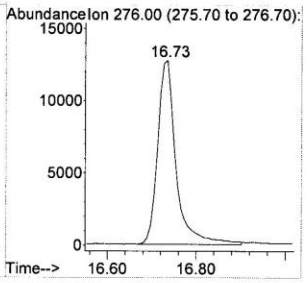
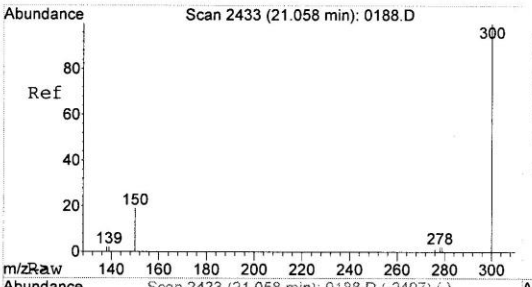


#22
Benzo(g,h,i)perylene
Concen: 200.00
RT: 16.73 min Scan# 1863
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:276 Resp: 36354

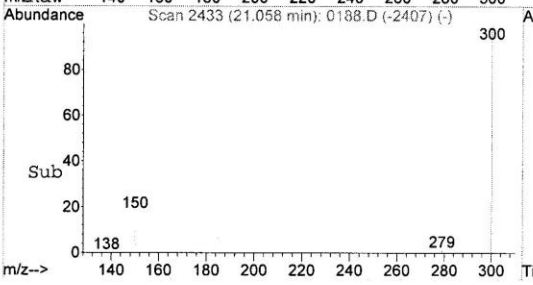






#23
Coronene
Concen: 200.00
RT: 21.06 min Scan# 2433
Delta R.T. 0.00 min
Lab File: 0188.D
Acq: 16 Jun 2009 18:07

Tgt Ion:300 Resp: 20652



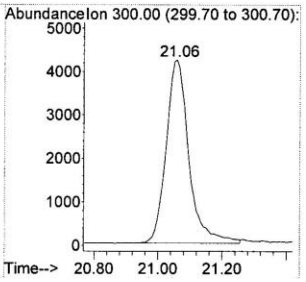
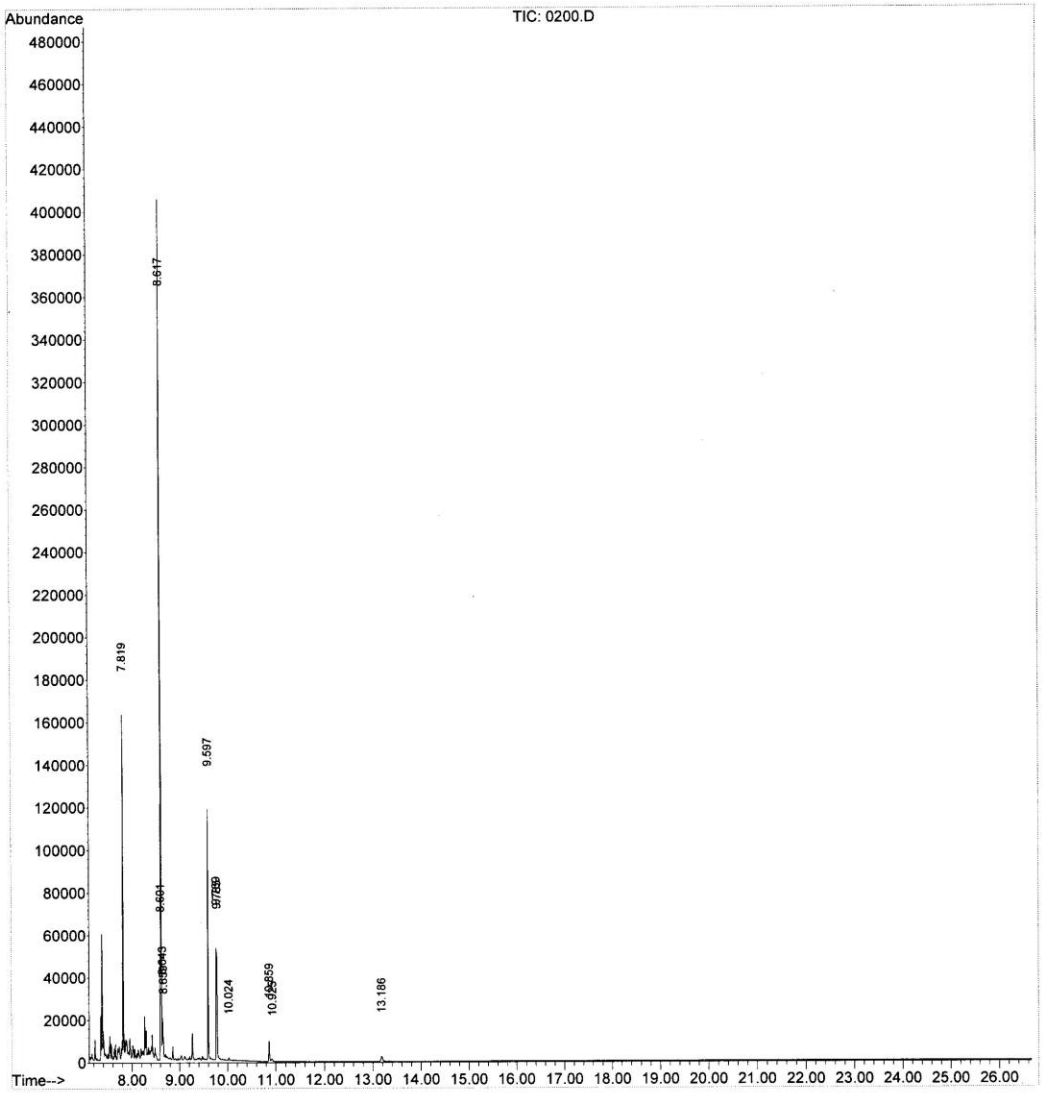


Figure IV. An example of the chromatogram for an IADN sample.

Quantitation Report (QT Reviewed)

Data Path : C:\MSDChem\1\DATA\
Data File : 0200.D
Acq On : 17 Jun 2009 00:26
Operator : YUNING
Sample : LH 01C 080928-DIL
Misc : S308C
ALS Vial : 38 Sample Multiplier: 1

Quant Time: Jun 18 16:06:37 2009
Quant Method : C:\MSDCHEM\1\METHODS\PAH_NEWCUT_JUNE.M
Quant Title : IADN PAH
QLast Update : Thu Jun 18 15:08:53 2009
Response via : Initial Calibration



PAH_NEWCUT_JUNE.M Tue Aug 04 12:15:38 2009 Page: 2

Quantitation Report (QT Reviewed)

Data Path : C:\MSDChem\1\DATA\
Data File : 0200.D
Acq On : 17 Jun 2009 00:26
Operator : YUNING
Sample : LH 01C 080928-DIL
Misc : S308C
ALS Vial : 38 Sample Multiplier: 1

Quant Time: Jun 18 16:06:37 2009
Quant Method : C:\MSDCHEM\1\METHODS\PAH_NEWCUT_JUNE.M
Quant Title : IADN PAH
QLast Update : Thu Jun 18 15:08:53 2009
Response via : Initial Calibration

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)
1) d-10 Anthracene	8.64	188	14623m	200.00		0.00
12) d12-Dibenz(a)anthracene	10.86	240	10919m	200.00		0.00
15) d12-Perylene	13.19	264	5500	200.00		0.00
System Monitoring Compounds						
5) d10-Phenanthrene	8.60	188	38010	400.85		0.00
Spiked Amount 200.000			Recovery = 200.43%			
10) d10-Pyrene	9.77	212	37545m	443.32	ng	0.00
Spiked Amount 200.000			Recovery = 221.66%			
Target Compounds						Qvalue
2) Acenaphthylene	0.00	152	0	N.D.	d	
3) Acenaphthene	0.00	154	0	N.D.	d	
4) Fluorene	7.82	166	55750m	750.01		
6) Phenanthrene	8.62	178	243130	2065.03		100
7) Anthracene	8.66	178	7918m	76.36		
8) Fluoranthene	9.60	202	78993m	867.46		
9) Pyrene	9.78	202	35344m	301.95		
11) Retene	10.02	219	451	12.58		69
13) Benz(a)anthracene	0.00	228	0	N.D.	d	
14) Chrysene + Triphenylene	10.92	228	908m	8.16		
16) Benzo(b)fluoranthene	0.00	252	0	N.D.	d	
17) Benzo(k)fluoranthene	0.00	252	0	N.D.	d	
18) Benzo(e)pyrene	0.00	252	0	N.D.	d	
19) Benzo(a)pyrene	0.00	252	0	N.D.	d	
20) Indeno(1,2,3-cd)pyrene	0.00	276	0	N.D.	d	
21) Dibenz(a,h)anthracene	0.00	278	0	N.D.	d	
22) Benzo(g,h,i)perylene	0.00	276	0	N.D.	d	
23) Coronene	0.00	300	0	N.D.	d	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

Figure V. An example of the chromatogram for an Autotune file.

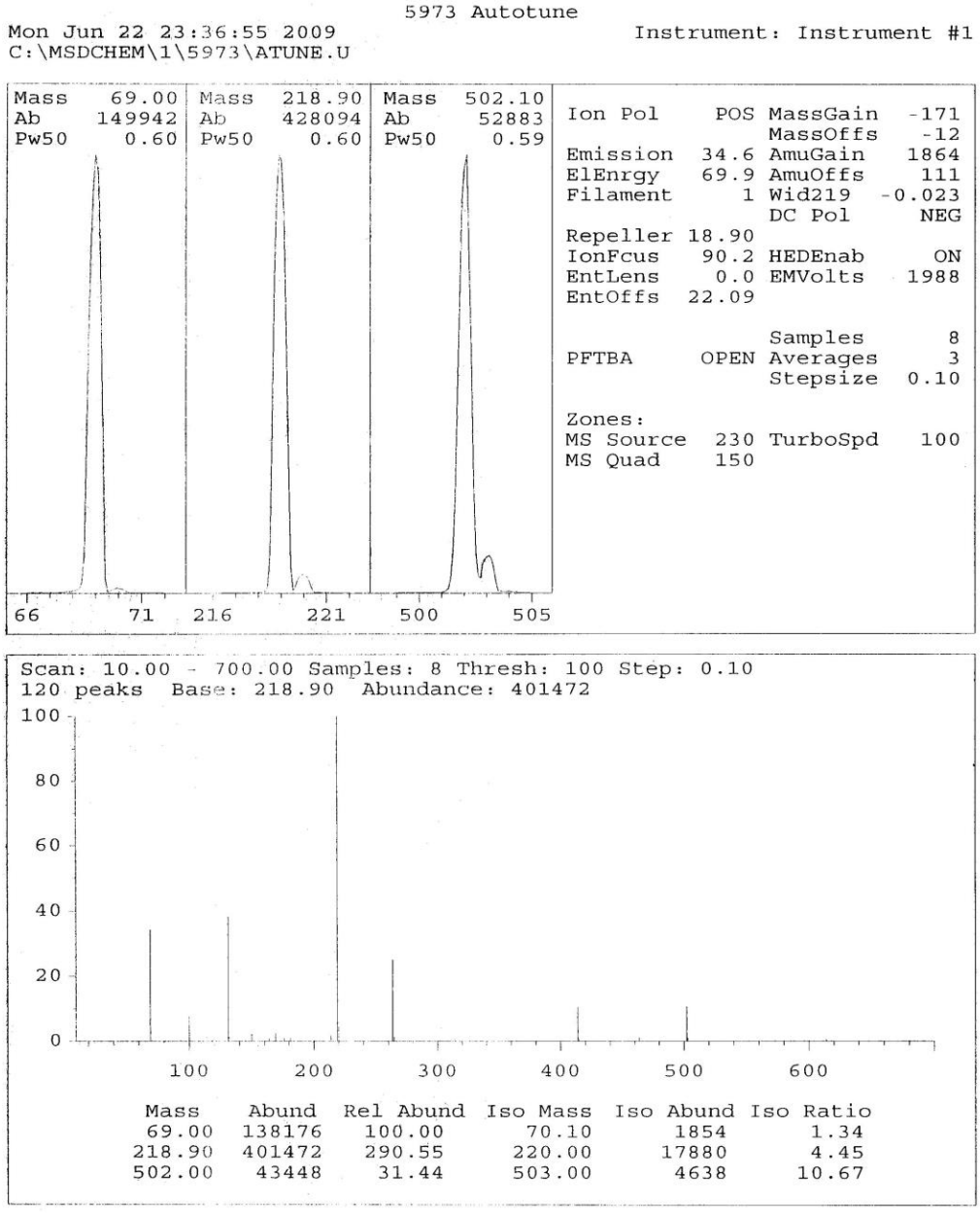
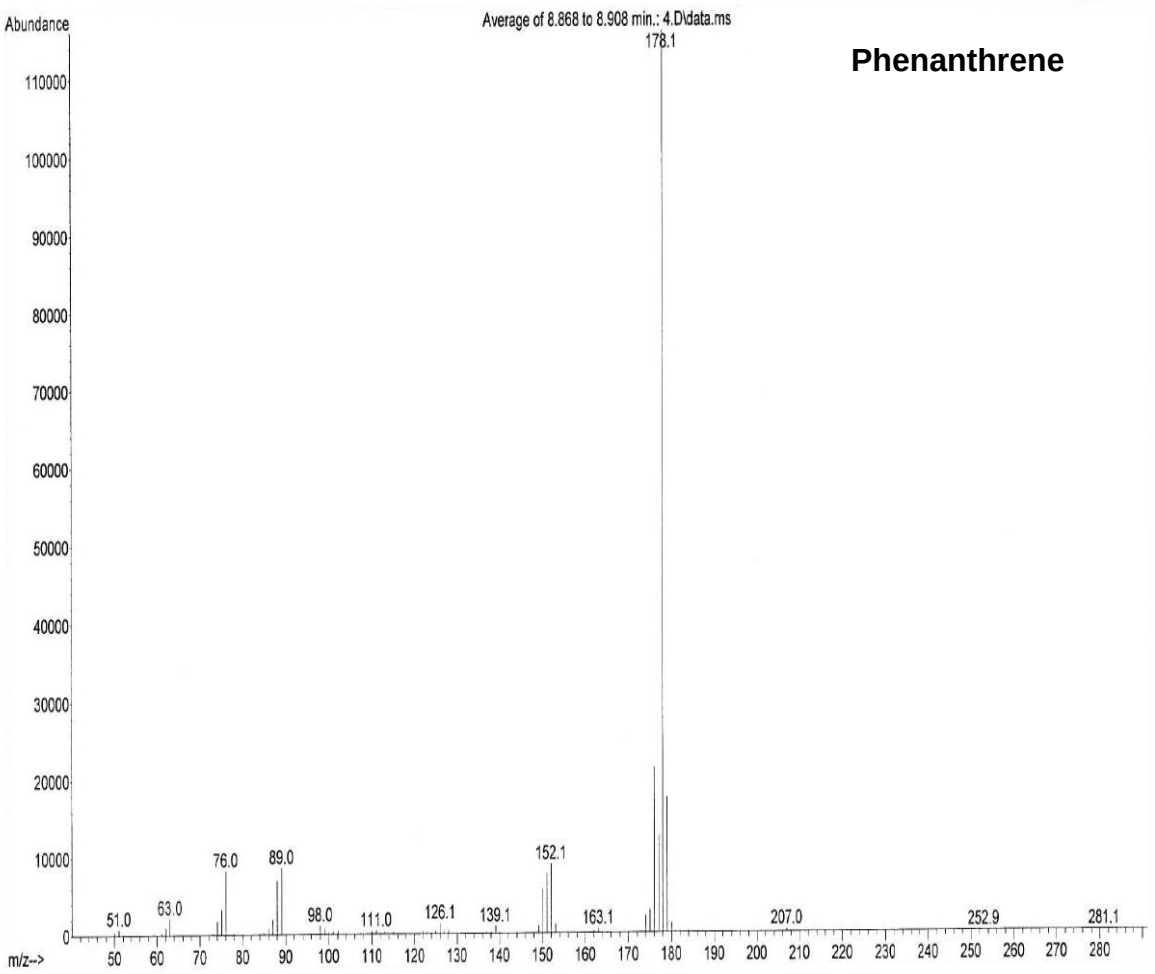
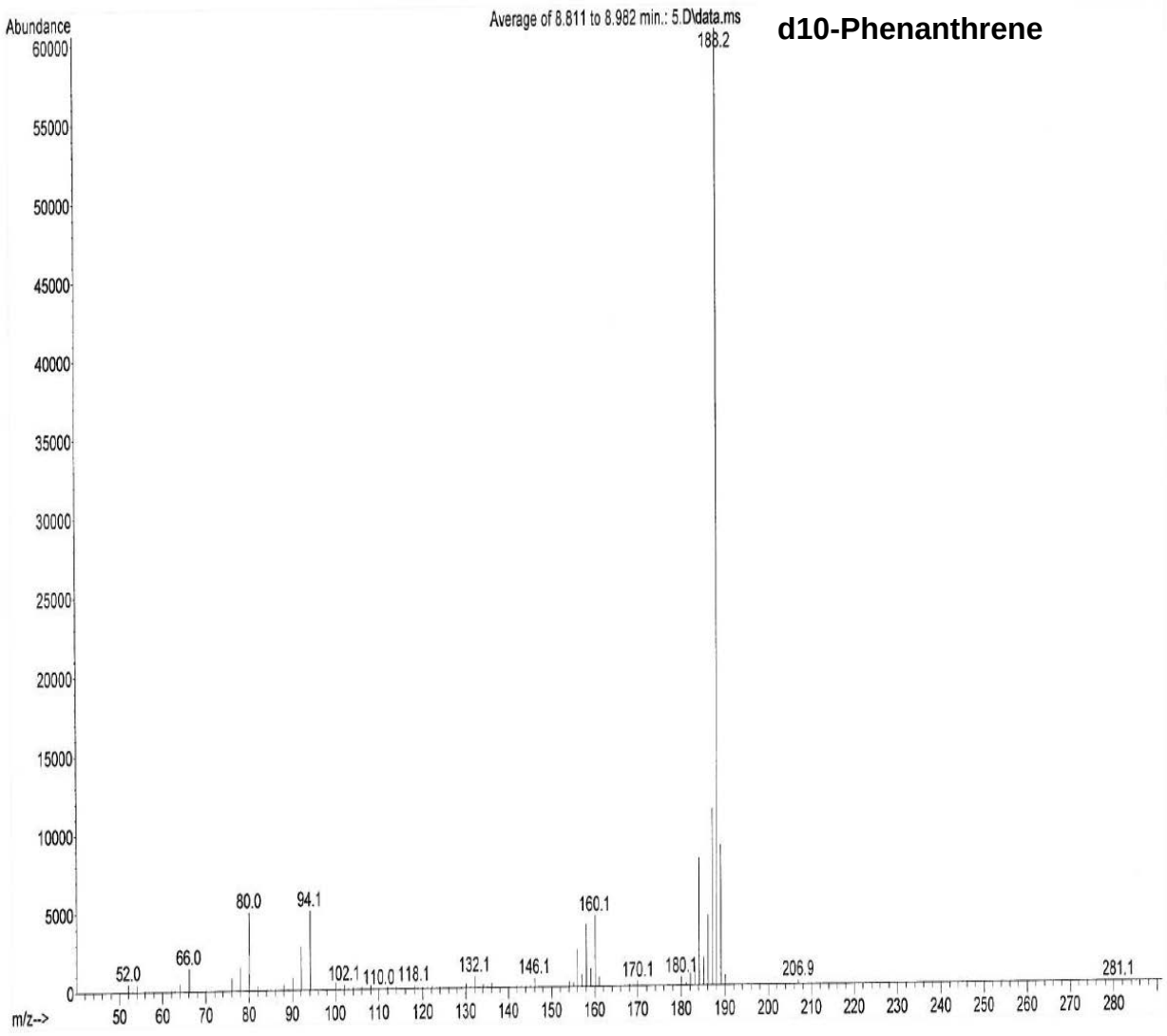


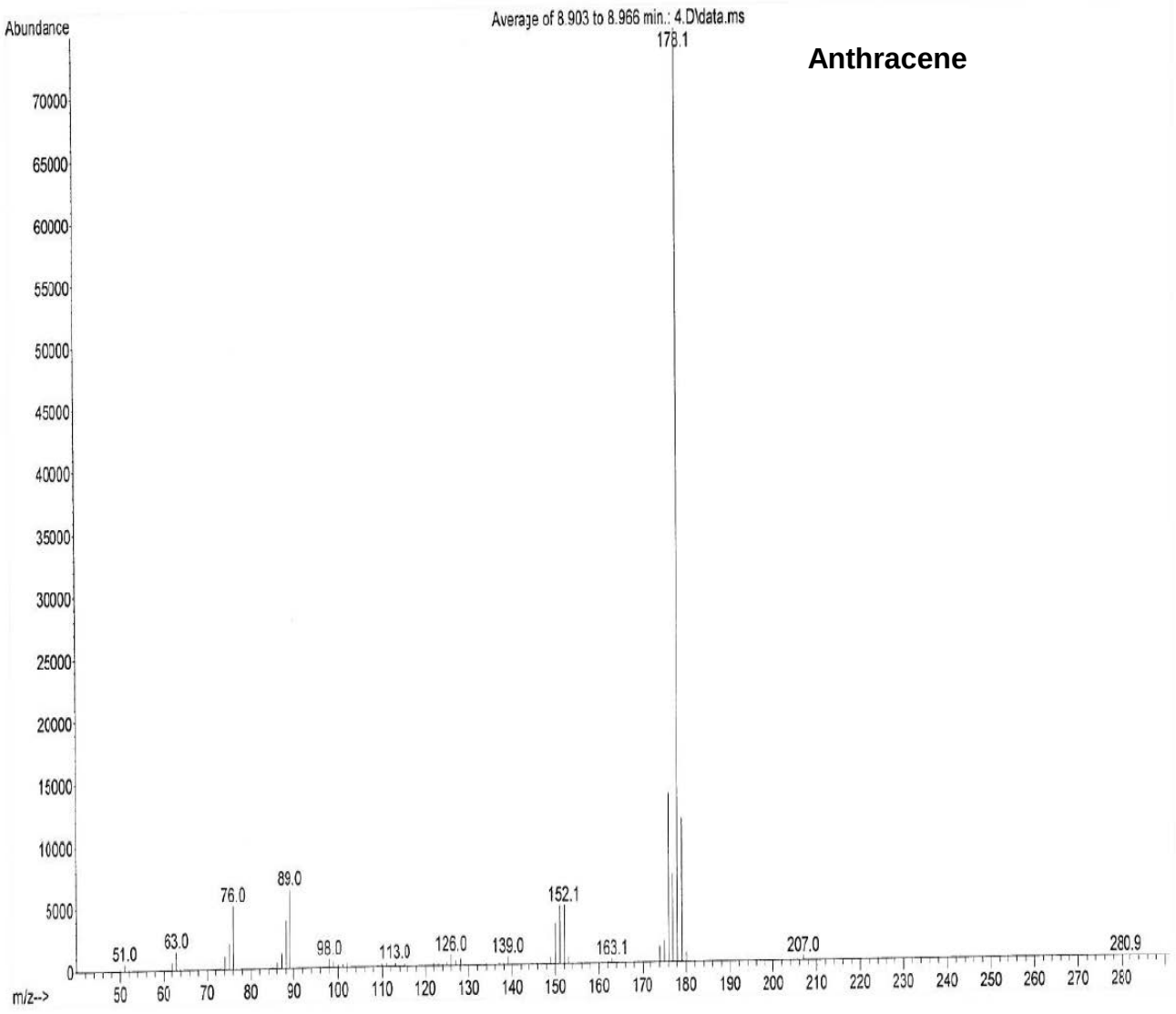
Figure VI. Mass spectra of Phenanthrene, d10-Phenanthrene, Anthracene,

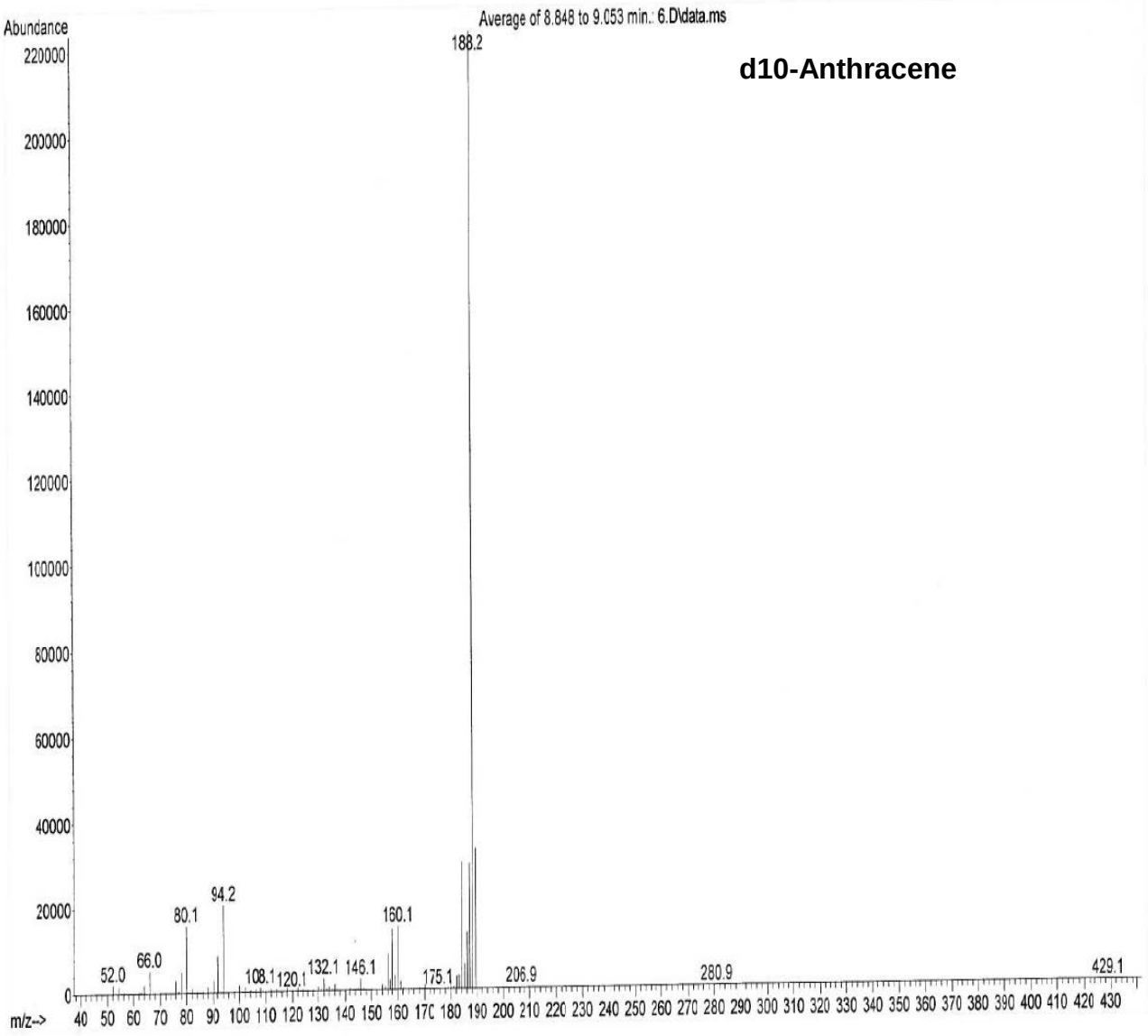
d10-Anthracene, Pyrene, d10-Pyrene, Benz[*a*]anthracene, and d12-Benz[*a*]anthracene.

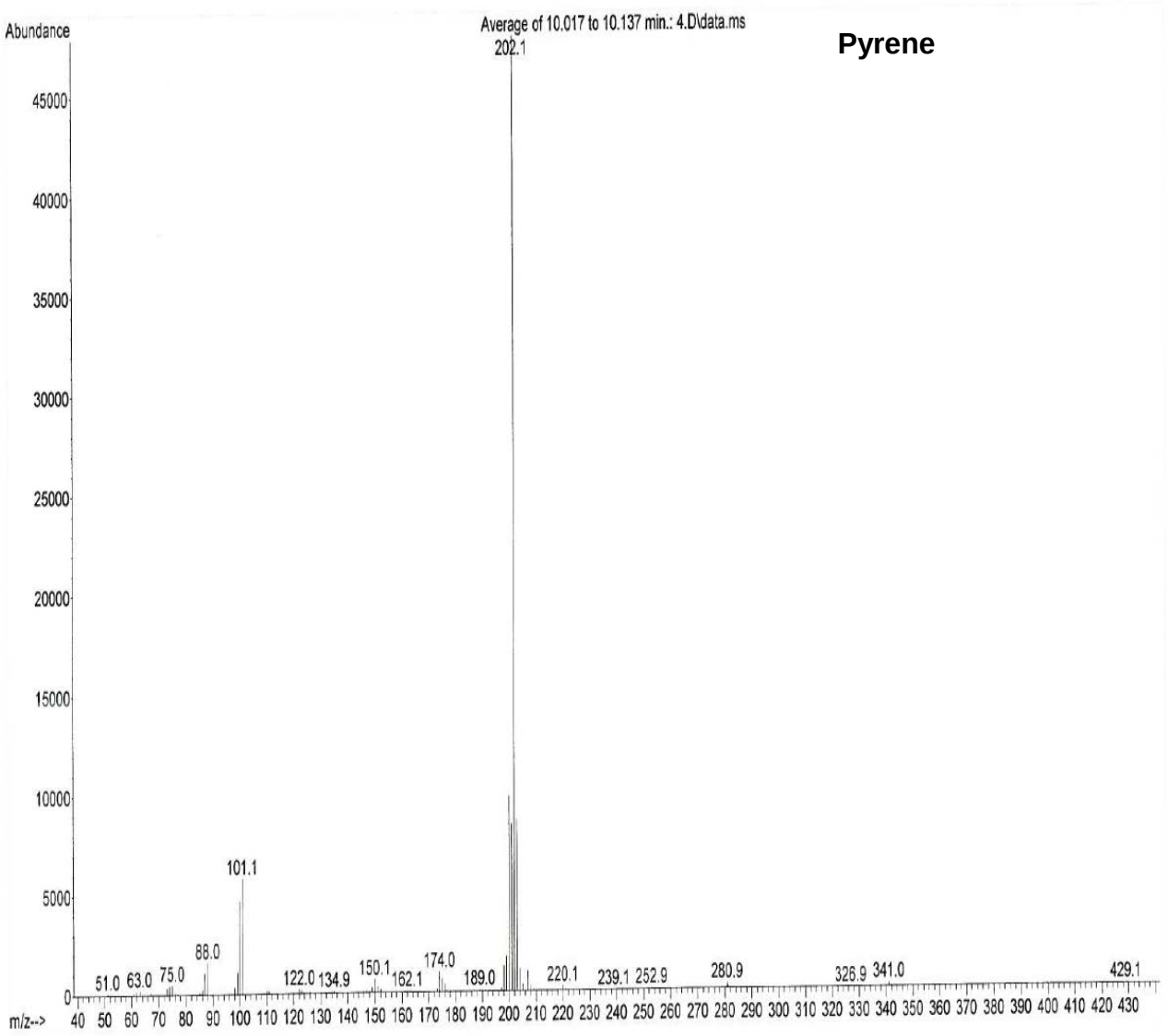


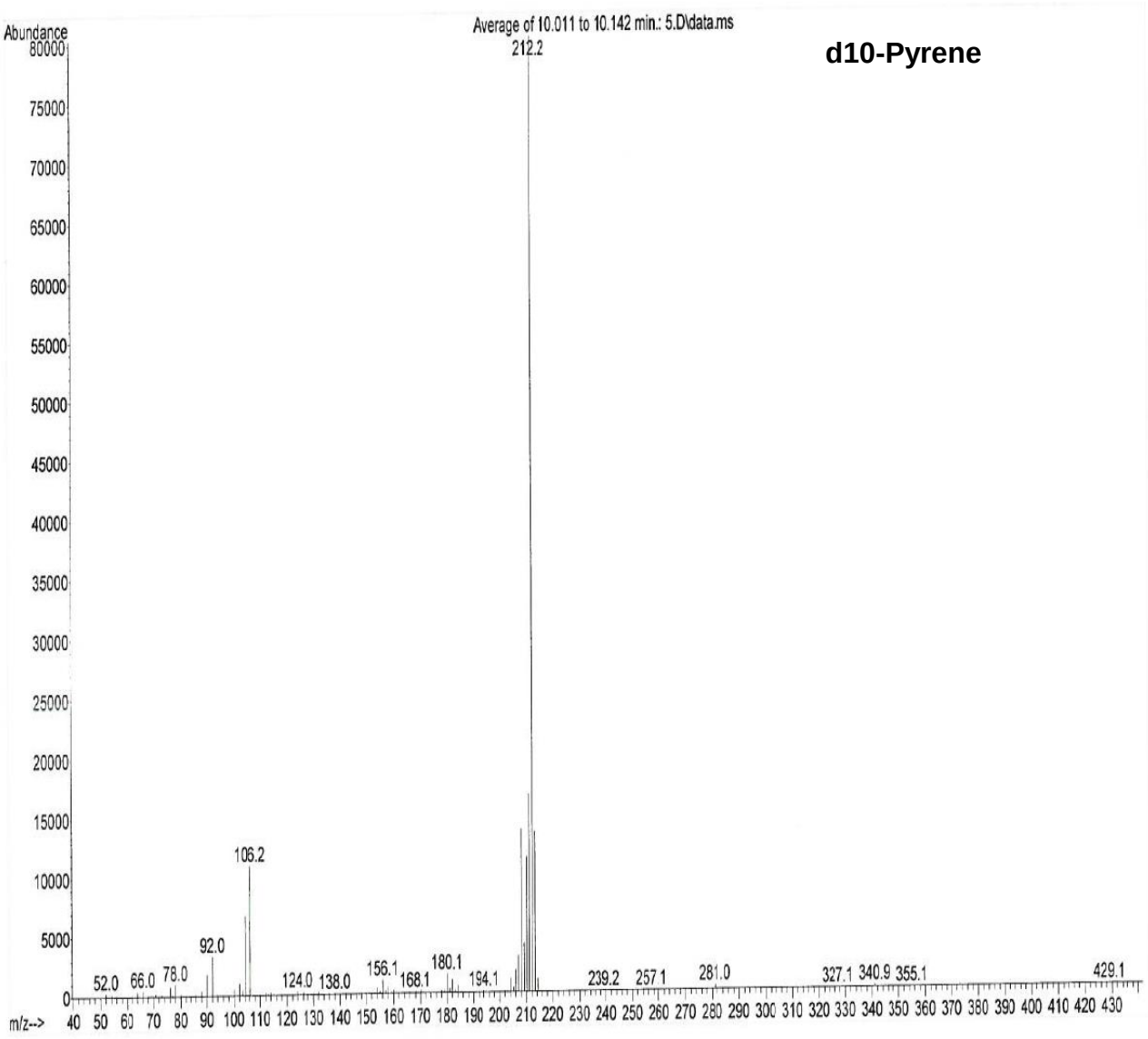


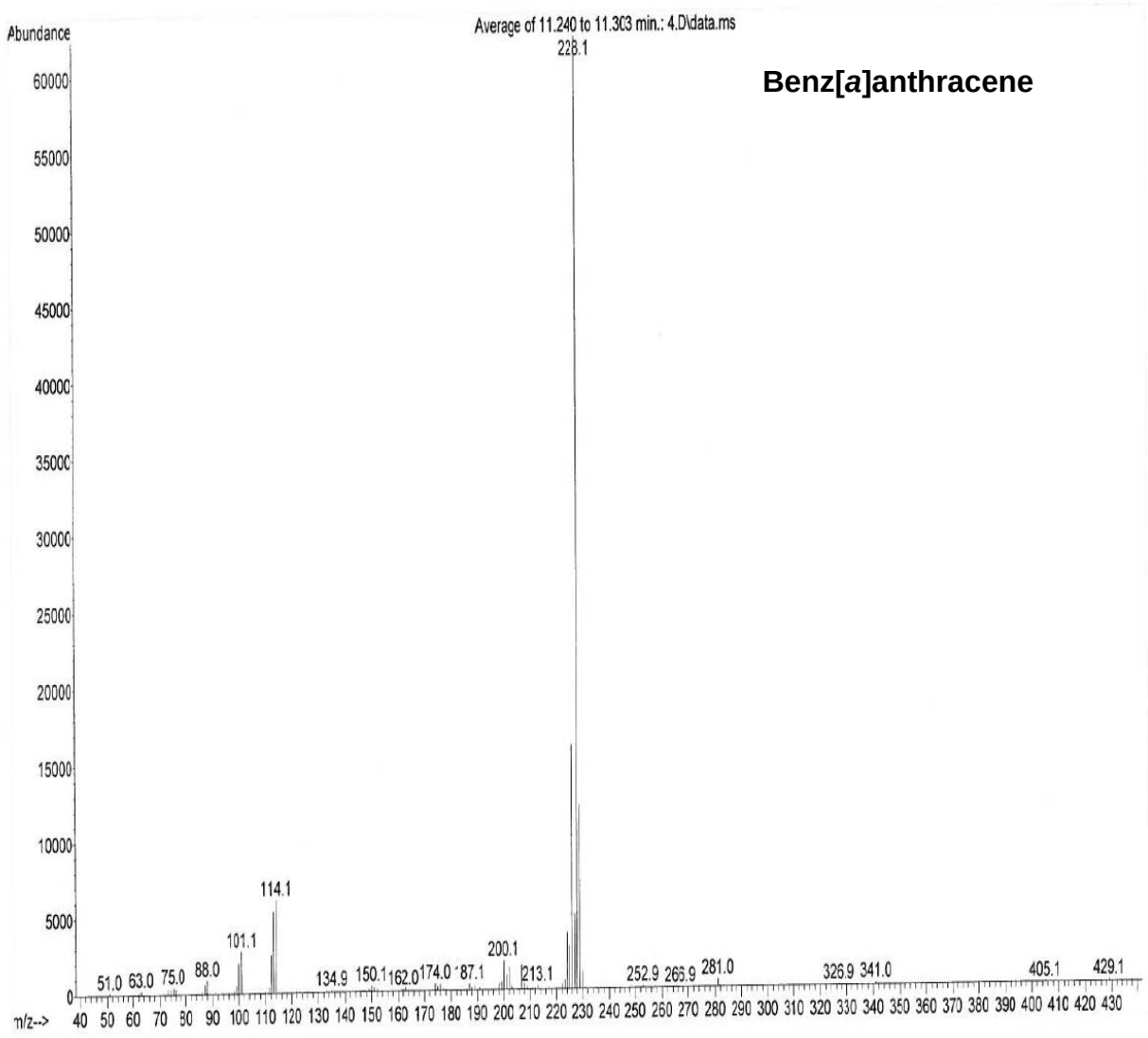
d10-Phenanthrene

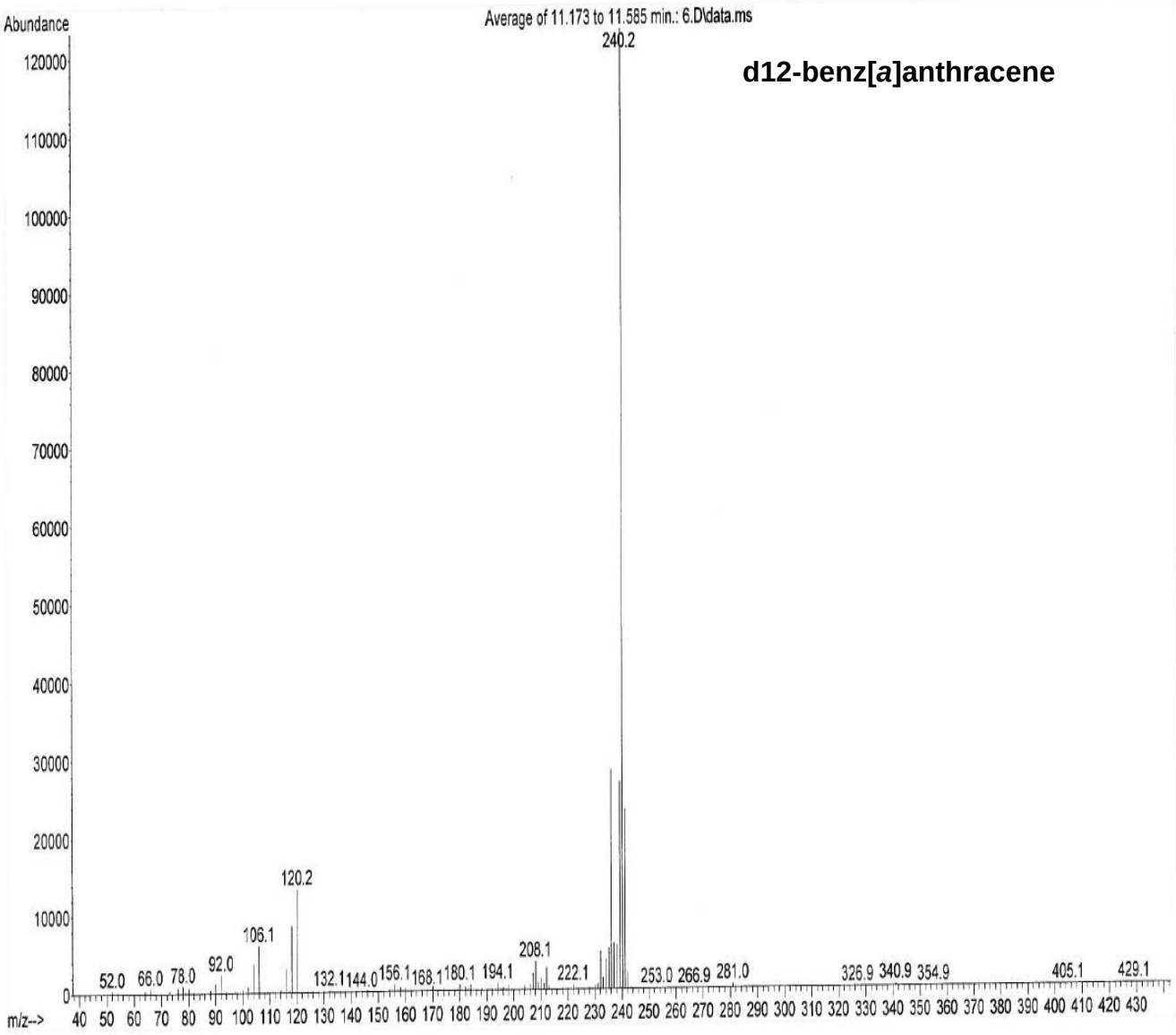

Average of 8.903 to 8.966 min.: 4.D\data.ms
Anthracene
Abundance
178.1
152.1
89.0
76.0
63.0
51.0
98.0
113.0
126.0
139.0
163.1
207.0
280.9
m/z-->


Abundance
Average of 8.848 to 9.053 min.: 6.D\data.ms
188.2
d10-Anthracene
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
94.2
80.1
160.1
66.0
52.0
108.1
120.1
132.1
146.1
175.1
206.9
280.9
429.1
m/z-->
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430


Abundance
Average of 10.017 to 10.137 min.: 4.D\data.ms
202.1
Pyrene
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
101.1
88.0
51.0 63.0 75.0
122.0 134.9
150.1
162.1
174.0
189.0
220.1
239.1 252.9
280.9
326.9 341.0
429.1
m/z-->
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430


Average of 10.011 to 10.142 min.: 5.D\data.ms
d10-Pyrene
Abundance
212.2
106.2
92.0
180.1
156.1
m/z-->


Average of 11.240 to 11.303 min.: 4.D\data.ms
Abundance
228.1
Benz[a]anthracene
114.1
101.1
200.1
m/z-->


Abundance
Average of 11.173 to 11.585 min.: 6.D\data.ms
240.2
d12-benz[a]anthracene
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
120.2
106.1
208.1
52.0 66.0 78.0 92.0 132.1 144.0 156.1 168.1 180.1 194.1 222.1 253.0 266.9 281.0 326.9 340.9 354.9 405.1 429.1
m/z-->
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430